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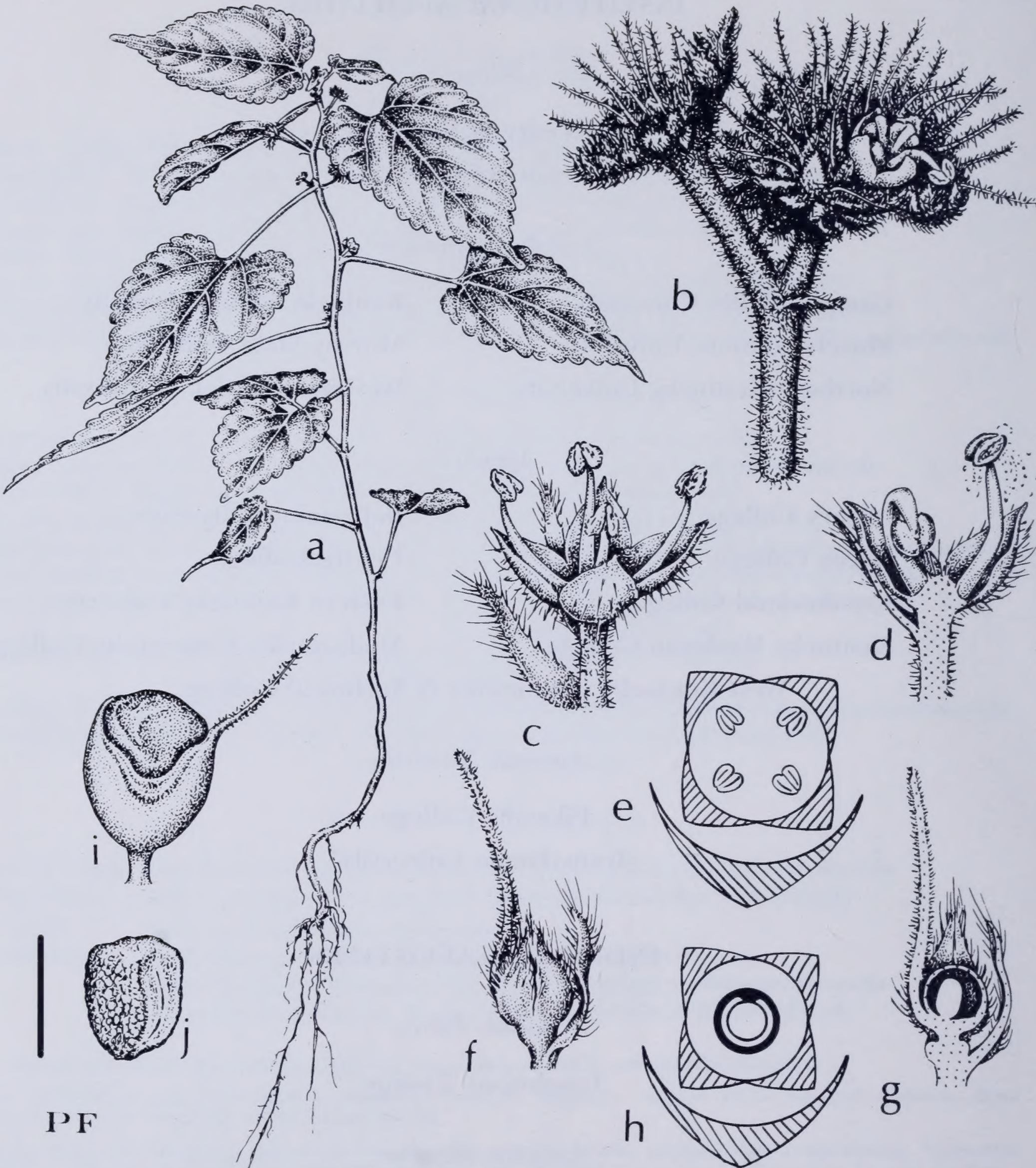

Figure 1. *Fatoua villosa.* **a.** Whole plant. **b.** Inflorescence. **c.** Staminate flower. **d.** Staminate flower, long section. **e.** Floral diagram of staminate flower. **f.** Pistillate flower. **g.** Pistillate flower, long section. **h.** Floral diagram of pistillate flower. **i.** Fruit. **j.** Seed. Bar = 1.5 cm for a; 0.5 mm for b; 1 mm for c, d, f, g–j: Drawings by Priscilla Fawcett; taken from Correll and Correll 1982; used by permission of the publisher.

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Spread of *Fatoua villosa* (Mulberry Weed; Moraceae) in North America

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ABSTRACT

Fatoua villosa (mulberry weed, Moraceae) is documented from 28 states and the District of Columbia in the United States. The species has spread through the horticultural trade to greenhouses and nurseries, from which it has escaped into gardens, lawns, and ruderal areas. A small percentage of the collections examined were from more natural settings in open forested sites or along streams. The species is illustrated and described, and maps are provided showing its spread in the 50 years since it was first collected in this country. First reports are provided for mulberry weed in the District of Columbia, Illinois, Minnesota, Oregon, Pennsylvania, and Wisconsin.

INTRODUCTION

Forty years ago, Thieret (1964) reported the discovery of a new weedy plant species for North America, *Fatoua villosa* (Thunb.) Nakai, commonly called "mulberry weed," "hairy crabweed," or "kuwa-kusa," a member of the Moraceae (mulberry family). Thieret cited information provided by Dr. Joseph Ewan, of New Orleans, that the species had been known in that city for about 15 years. Since that time, the species has spread across North America, and literature reports exist for its presence in 26 states in the United States.

The genus *Fatoua* was described by Gaudichaud (Freycinet 1830). Two or three species of the genus are native to Australasia (Chew 1989; Hutchinson 1967; Rohwer and Berg 1993; Zhou and Gilbert 2003) and an additional species is found in Madagascar (Berg 1977; Leandri 1948). *Fatoua villosa* (Figure 1) was first described by Thunberg (1784) as *Urtica villosa* (Urticaceae); the epithet was transferred to the genus *Fatoua* by Nakai (1927). Synonyms include *Urtica japonica* Thunberg (1784), *Fatoua japonica* Blume (1856), and *Boehmeriopsis pallida* Komarov (1901). Miquel (1869) published the only monograph of the genus to date, treating it as a member of the Urticaceae. The first treatment of *Fatoua* as a member of Moraceae was by Gaudichaud (Freycinet 1830), who described two additional possible synonyms, *F. pilosa* and *F. aspera*. Placement of the genus in Moraceae is supported by Yamazaki (1982), who showed that seed development patterns in *Fatoua* follow those found in other Moraceae and is unlike that of Urticaceae.

Mulberry weed is native to China (Li 1986), Japan (Ohwi 1965), Korea (Komarov 1901; Lee 1989), Okinawa (Walker 1976), Ryukyu Islands (Walker 1976), Taiwan (Liu and Liao 1976), and Tonkin (Reed 1977). In addition to reports from the continental United States, which will be discussed below, mulberry weed has been reported as an introduced weed in the Bahamas (Correll and Correll 1984), Corsica (Jeanmonod 2000), Finland (Kuitunen and Lahtonen 1994), Hawaii (Yatskievych and Raveill 2001), and Puerto Rico (Liogier 1997; Liogier and Martorell 2000). In its native range, mulberry weed is considered weedy in

cultivated or grassy fields, along roadsides, on trailsides, in open woods, and in rocky areas (Holm et al. 1979; Ohwi 1965; Randall 2002; Walker 1976; Zhou and Gilbert 2003). As an introduced weed, it has been found in greenhouses, nurseries, flowerbeds, potted plants, and botanical gardens; a few records are from woodlands, riverbeds, and forest edges and fencerows.

Fatoua villosa is an herbaceous, erect, taprooted annual with colorless sap without latex. Stems of the species are 10–90 cm in height, may be simple to much branched, and range in color from green to maroon red; they are thinly to densely short hairy. In general aspect, the species greatly resembles taxa of Urticaceae and, indeed, when first encountered is often mistakenly identified as a member of that family. Its leaves are alternate, thinly scabrous, broadly ovate to lanceolate, 3–10 × 1–5 cm, toothed, cordate to truncate at the base, acute to acuminate at the apex; petioles range from 0.5–6 cm; stipules are small, free, and early-deciduous; venation in the leaves is palmate to pinnate. Punctate cystoliths, composed of calcium carbonate, are present in the leaves. Inflorescences are axillary, condensed cymes that are often nearly capitate, 4–7 mm wide, on peduncles that vary from very short to 2–3 cm in length; each inflorescence is subtended by a small bract. Plants are monoecious, with staminate and pistillate flowers in each inflorescence; inflorescences lower on the stem contain predominately pistillate flowers; the further up the stem the inflorescence is positioned, the larger the percentage of staminate flowers. Among the fertile pistillate flowers can be found staminate flowers with morphologically distinct pistils (pistillodes) without ovules. The calyx is 4-lobed, pubescent, yellowish in staminate flowers, and green in pistillate flowers; the corolla is absent. Functional pistils are bicarpellate and unilocular; the style is lateral and appears unbranched, but has a very small aborted style branch positioned at the base of the larger functional style; there is a single pendulous ovule. Staminate flowers contain four exserted stamens positioned alternately with the calyx lobes. Fruits are achenes, three-angled to nearly globose to flattened, 1–1.1 mm long, buff to dark brown, with whitish raised ridges; they are mostly dropped near the mother plant, but are also explosively expelled up to 4′—and probably farther, especially from plants growing as weeds in flower baskets hanging over greenhouse benches. Seeds have a small embryo, with endosperm. (Description compiled from specimens and the following sources: Miller and Wood 2003; Neal 1998; Reed 1977; Rohwer and Berg 1993; Sanders 1996; Thieret 1964; Wu and Kuo-Huang 1997; Wunderlin 1997; Yamazaki 1982; Yatskievych and Raveill 2001; Zhou and Gilbert 2003). Known chromosome counts are $n = 13$ (Kondo and Miller 1973) and $2n = 26$ Li (1986). Plants flower from July through October in outdoor settings in the north, August through November in the south, and through the summer and winter in deep south and greenhouse settings.

Swain and Downum (1989, 1990) showed that mulberry weed contains biologically active compounds, furanocoumarins, that are phototoxically active toward some test organisms, such as the bacterium *Escherichia coli.*

Control of the species as a weed has been extensively studied by Penny and Neal (1999a, 1999b). Pre-emergence control is most effectively obtained by use of products containing oxyflourfen and oxadiazon; dinitroaniline herbicides differed in effectiveness. Penny and Neal (2003) showed that seed burial and mulch were 90% effective at inhibiting germination, since germination requires light. Low or high temperatures may also affect germination and development, since the species grew best at temperatures between 15–38°C (Penny 2000). Postemergence control is most effective using products containing paraquat, glyphosate, and glufosinate and perhaps also diquat or pelargonic acid (Penny 2000).

In North America, mulberry weed is reported by Wunderlin (1997) from 17 states in the United States, and by Kartesz and Meacham (1999) and the USDA Plants Database (USDA, NRCS 2004) for 18 states. Additional reports in the literature bring the number to 26 states (Table 1). No reports are known of the species in Canada. It appears that the species is being spread through the horticulture industry, since many early reports are of the species as a weed in greenhouses, from which it presumably spreads by means of bedding plants, nursery stock, potting soil, or bedding mulch (Miller and Wood 2003; Penny 2000;

Table 1. States in the United States from which *Fatoua villosa* has been reported in published literature or observed as herbarium specimens. Listed are the source citations, the year of the earliest known collection examined during the course of this study, and the number of counties/parishes from which the species is reported (specimens and undocumented literature reports).

State	Literature source(s)	Earliest collection	Number of counties/parishes
Alabama	Massey 1975	1967	8
Arkansas	Smith 1994; Sundell 1986	1985	3
California	Hrusa et al. 2002; Randall 1997; Sanders 1996; Baldwin et al. 2004	1983	7
District of Columbia		1994	1
Florida	DuQuesnay 1974; Massey 1975; Wunderlin and Hansen 2004	1970	17
Georgia	Jones and Coile 1988; Massey 1975	1965	11
Illinois		1982	2
Indiana	Maxwell and Thomas 2003; Wunderlin 1997	1995	1
Iowa	Cusick 2002	2000	1
Kentucky	Browne and Athey 1992; Taylor 1994	1983	6
Louisiana	MacRoberts 1989; Massey 1975; Thieret 1964; Thomas et al. 1980	1950	18
Maryland	Wunderlin 1997	1981	4
Massachusetts	Miller and Wood 2003	1994*	1
Michigan	Reznicek 2001	2001	1
Minnesota		1998	1
Mississippi	Carter et al. 1990; Massey 1975	1972	9
Missouri	Wunderlin 1997; Yatskievych and Raveill 2001	1972	7
New York	Miller and Wood 2003	2002	1
North Carolina	Massey 1975; Neal 1998	1973	7
Ohio	Vincent 1993	1979	11
Oklahoma	Taylor et al. 1996; Taylor and Taylor 1981	1979	7
Oregon		2000	1
Pennsylvania		1989	1
South Carolina	Anonymous 2004b; Wunderlin 1997	1975	7
Tennessee	Kral 1981; Anonymous 2004c	1970	4
Texas	Jones et al. 1997; Lipscomb 1984	1974	9
Utah	Welsh et al. 2003	1997	1
Virginia	Wright 1988	1987	4
Washington	Anonymous 1996; Anonymous 2004d	1995*	1
West Virginia	Wunderlin 1997	*	
Wisconsin		2002	1

* = not seen.

Penny and Neal 1999a, 1999b; Sanders 1996; Taylor 1994; Taylor et al. 1996; Vincent 1993; Welsh et al. 2003; Wunderlin 1997; Yatskievych and Raveill 2001). Penny (2000) and Penny and Neal (1999a, 1999b) report 50% of nurseries surveyed in 1997 and 75% of nurseries surveyed in 1998 were infested by this weedy species. One or two generations can occur in a growing season (Penny 2000; Penny and Neal 1999a, 1999b).

This study was undertaken to determine the extent to which *Fatoua villosa* has spread in North America since its introduction, and if any pattern can be discerned regarding its spread.

MATERIALS AND METHODS

In addition to field work to find the species, herbarium specimens were examined from the following herbaria (acronyms from Holmgren 2004): A, AUA, BALT, BAYLU, BH, BHO, BKL, BRIT, C, CDA, CLEMS, CM, DAV, DOV, F, FTG, GA, GH, GMUF, ID, ILL, ILLS, ISC, JEF, KNK, LAF, LSU, MICH, MIN, MISS, MISSA, MO, MONTU, MU, NA, NBYC, NCU, NHA, NLU, NO, NY, OCLA, OKL, OS, OSH, RM, SMU, TENN, TEX, UAM, UCR, UNA, UNLV, URV, US, USCH, USF, VDB, VPI, VT, WIS, WSU, WTU, WVA.

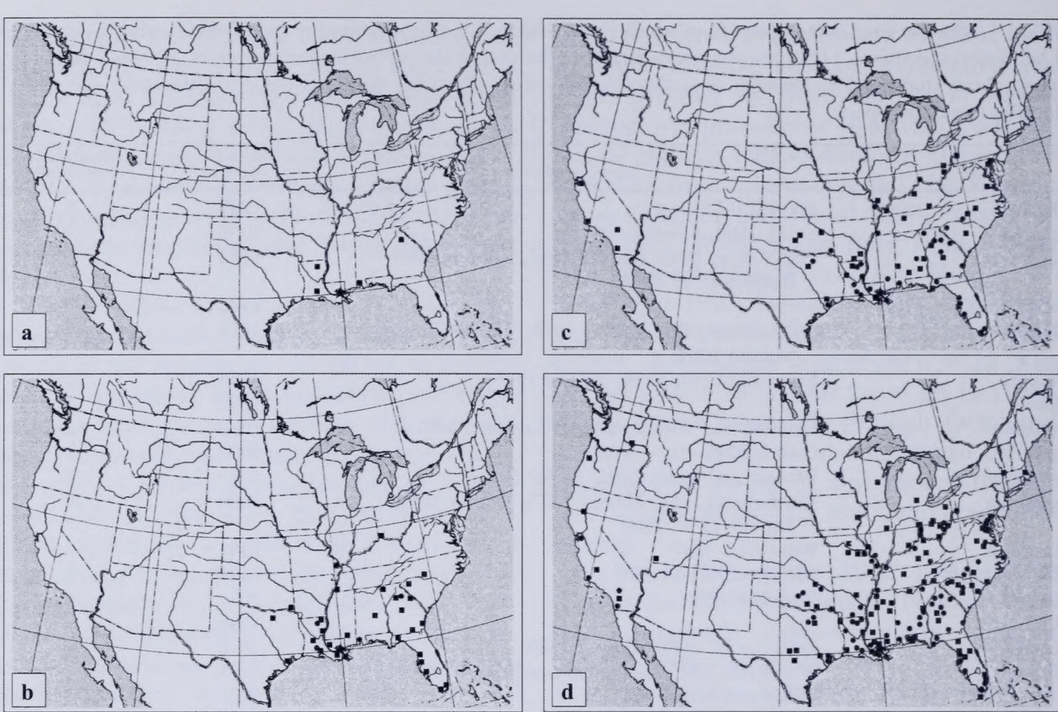

Figure 2. Distribution of *Fatoua villosa* in North America from its initial discovery in Louisiana to the present. Each square (■) represents the first report of the species for a particular county. Dots (●) represent these first reports carried over from previous map(s). **a.** 1950–1969. **b.** 1970–1979. **c.** 1980–1989. **d.** 1990–2004.

RESULTS AND DISCUSSION

A total of 495 herbarium specimens were examined during the course of this study. Collections were seen from 134 counties in 28 states, and the District of Columbia (Appendix 1). An additional 21 counties are listed in literature reports for which no voucher documentation was found. Of the counties represented by specimens, 17 were represented only as greenhouse weeds and not by specimens from sites out-of-doors.

The earliest known specimen of mulberry weed from North America is from New Orleans, Orleans Parish, Louisiana, collected in 1950 (*G. P. DeWolf s.n.* [NO]). Table 1 shows the dates of the earliest known specimen from each state, as well as the number of counties/parishes from which the species is known for each state. The state with the largest number of counties/parishes from which mulberry weed has been collected (specimens plus additional literature reports) is Louisiana (18), followed by Florida (17), Georgia (11), and Ohio (11). Even though the species was reported by Wunderlin (1997) for West Virginia, no specimen could be located to document its occurrence in that state.

Based on the herbarium specimens examined, beginning with Louisiana, from which the earliest collection is known (1950), the species appeared in the 1960's in Georgia (1965) and Alabama (1967). In the 1970's it turned up in Florida (1970), Tennessee (1970), Mississippi (1972), Missouri (1972), North Carolina (1973), Texas (1974), South Carolina (1975), Oklahoma (1979), and Ohio (1979). In the 1980's, the species was found in Maryland (1981), Illinois (1982), California (1983), Kentucky (1983), Arkansas (1985), Virginia (1987), and Pennsylvania (1989). In the 1990's specimen were collected in DC (1994), Massachusetts (1994), Indiana (1995), Washington (1995), Utah (1997), and Minnesota (1998). Since 2000, the species has been collected in Oregon (2000), Iowa (2000), Michigan (2001), Wisconsin (2002), and New York (2002).

When the distribution of the specimens is mapped (Figure 2), an interesting pattern emerges with regard to the spread of the species in the last 50 years. Beginning with the earliest known collection, in New Orleans in 1950, collections gradually radiate out to the southeast, east, northeast, north, northwest, and west. Over the next 50 years, the range of the species gradually increases until the present-day extent is reached. It cannot be discerned from the available data whether multiple introductions occurred into the United States, or if the species spread from an initial introduction site in New Orleans. The source of the initial introduction is also unknown.

Spread of the species does seem to result from horticultural practices. Of the herbarium specimens studied, 50% are from gardens or flower beds, 13% are from nurseries, 12% are from greenhouses, 7% are from lawns, 5% are from potted plants, and 2% are from other horticultural sources (compost piles, mulch, soil piles). Of the remainder, sites from which specimens were collected included old fields, fencerows, cracks and crevices, railroad yards, roadsides, waste places, and dirt or gravel parking lots. Only 6% were from sites away from cultivated areas, where the populations could be considered "naturalized."

CONCLUSIONS

Fatoua villosa is an increasingly common and widespread weed in the continental United States. It is apparently spreading by means of both plants and propagules through the horticultural trade. The species may also be spreading by other means, e.g., import of seeds obtained through seed indices/lists (Anonymous 2004a). While most of the known specimens are from horticultural sites, such as greenhouses, nurseries, and gardens, the species has spread from these areas into adjacent ones, such as lawns, fencerows, and other ruderal areas. From these habitats, mulberry weed has seemingly spread in some areas to more natural settings, such as forest edges and along streams. Since the species is able to survive and spread in both open and shaded habitats, it may spread from horticultural and ruderal settings into more undisturbed sites, especially in open forests, a habitat it occupies in its native range.

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APPENDIX

Selected Specimens Examined

(only 1 specimen per county; * = greenhouse weed)

ALABAMA: Chambers County, *L. Dalrymple s.n.* (AUA); Conecuh County, *A.R. Diamond 3650* (GA, NLU); Elmore County, *E.R. Burns s.n.** (AUA); Lee County, *J.M. Moffett s.n.* (TENN); Madison County, *C.T. Bryson 17529* (USCH); Mobile County, *K.E. Rogers 1819 & LeLong* (NCU); Pike County, *A.R. Diamond 5330* (AUA); Sumter County, *R.D. Thomas 100747 & Pittman* (NLU, NY).

ARKANSAS: Drew County, *E. Sundell 6834 & Guldin* (BRIT, NLU, UAM); Pulaski County, *E. & M. Sundell 12049* (NLU, UAM); Union County, *R.D. Thomas 107891 et al.* (MO, TENN).

CALIFORNIA: Alameda County, *R.D. Raabe & Strother s.n.** (A); Kern County, *Lapp et al. s.n.* (CDA); Riverside County, *A.C. Sanders 15832* (UCR); San Bernardino County, *M. Cohen s.n.* (CDA); San Diego County, *F. McCutcheon s.n. & Avery* (CDA); Santa Barbara County, *K. Cheesman s.n.* (CDA).

DISTRICT OF COLUMBIA: Washington, *S. McNaull 141 & Csiba* (MU).

FLORIDA: Broward County, *M.A. Vincent 10998 & Hickey* (MU); Citrus County, *F. Damian s.n.* (GH, NCU); Dade County, *J. Popenoe 867* (FTG, NCU); Franklin County, *L.C. Anderson 8671* (MO); Hillsborough County, *J.M. Kunzer 556* (USF); Leon County, *R.K. Godfrey 72357* (GA, MO, NCU, NY, TENN); Monroe County, *J. Popenoe 1979* (FTG, NCU); Pasco County, *D. DuQuesnay s.n.* (A, CM, FTG, GA, MIN, NCU, NLU, SMU, USF); Sarasota County, *J. Popenoe 1979* (USF).

GEORGIA: Bibb County, *J.R. Allison 3514* (GA); Clarke County, *W.H. Duncan s.n.** (GA, LSU); Columbia County, *J. Allison 942* (BH, GA, MO, NCU); Fulton County, *R.D. Thomas 101286 et al.* (NLU); Glynn County, *W.H. Duncan 30555* (GA, MO); Gwinnett County, *S.B. Jones 22525* (GA, NCU); Jones County, *W.H. Duncan 30564* (GA, WIS); Lowndes County, *W.R. Faircloth 6736* (GA, MO, NCU); Sumter County, *R.A. Norris 6740* (GA, NLU); Walker County, *W.H. Duncan 30647* (MO).

ILLINOIS: Champaign County, *S.R. Hill 33109* (BRIT, MU, NY, TEX, VT); Jackson County, *A.C. Koelling 6968* (ILL).

INDIANA: Floyd County, *Maxwell s.n.* (JEF).

IOWA: Muscatine County, *A.W. Cusick 35602* (MU, OSH).

KENTUCKY: Adair County, *J.W. Thieret 60461* (KNK);

Campbell County, *J.W. Thieret 57160* (KNK); Jefferson County, *M. Medley 9311-83* (KNK); Madison County, *M.A. Vincent 7463* (KNK, MO, MU).

LOUISIANA: Caddo Parish, *R.D. Thomas 166976 & Raymond* (NLU); Calcasieu Parish, *R. Neyland 1143* (LSU); Concordia Parish, *R.D. Thomas 24566* (NCU, NLU, TENN); East Baton Rouge Parish, *S. Tucker s.n.* (LAF, LSU, NLU, TEX); Iberia Parish, *R.D. Thomas 19706 et al.* (NLU); Jefferson Parish, *T. Zebryk 3297* (NLU); Lafayette Parish, *J.W. Thieret 16171* (A, LAF, SMU); Lafourche Parish, *R.D. Thomas 79963 et al.* (BRIT, NCU, NLU); Lincoln Parish, *A.W. Boyd 3208* (NLU); Morehouse Parish, *R.D. Thomas 56634 & Pias* (NLU); Natchitoches Parish, *W.C. Holmes 4001* (NLU, NO); Orleans Parish, *J. Ewan 23056* (NO, WIS); Ouachita Parish, *R.D. Thomas 17629* (GA, ILL, NLU, USF, WTU); Rapides Parish, *E. McWilliams M539024* (NLU); St. Charles Parish, *G. Montz 5294* (BRIT, LSU, LAF, NLU, NO); St. John Parish, *G.N. Montz 5946* (LSU, NLU, NO); St. Tammany Parish, *T. Zebryk 3301* (NLU); Tangipahoa Parish, *G.N. Montz 8932* (LSU, NLU, NO).

MARYLAND: Baltimore County, *C.F. Reed 111095** (MO); Harford County, *C.F. Reed 126949** (MO); Howard County, *J. Duke s.n.* (NA); Montgomery County, *F.G. Meyer 22475* (A).

MICHIGAN: Jackson County, *A.A. Reznicek 11300* (DOV, GH, ILLS, MO, MU, OSH).

MINNESOTA: Anoka County, *B.A. Addison s.n.** (MIN).

MISSISSIPPI: DeSoto County, *C.T. Bryson 18056 et al.* (NLU, TENN); Forrest County, *K.E. Rogers 7902* (NCU); Franklin County, *C. Havran 1238* (MISS, MISSA); Grenada County, *C.T. Bryson* (TENN); Lafayette County, *M.B. Huneycutt s.n.* (MISS); Monroe County, *J.R. MacDonald 9258 et al.* (DOV); Oktibbeha County, *C.T. Bryson 19176 & Bryson* (CM, DOV, MO); Pearl River County, *C.T. Bryson 16933 & Sudbrink* (MISS); Washington County, *C.T. Bryson 15655* (GA, KNK, MO, NLU, TENN, UNLV).

MISSOURI: Boone County, *P.M. McKenzie 1629* (MO); Butler County, *S. Hudson 956* (MO); Cape Girardeau County, *T.E. Brooks s.n.* (MO); Cole County, *T.E. Smith 3605* (MO); St. Louis County, *M.A. Vincent 6443* (MO, MU).

NEW YORK: Rensselaer County, *N.G. Miller 15565* (GH, MU).

NORTH CAROLINA: Brunswick County, *M.A. Vincent 8681* (BAYLU, C, DAV, ID, ILLS, MU, NCU, OSH, TEX); Cherokee County, *E. Lunsford s.n. & Morrow* (NCU); Dare County, *M.A. Vincent 9437* (MU); Durham County, *C.F. Reed 116878** (MO); Iredell County, *J.B. Nelson 2809 & Wnek* (NCU); Mecklenburg County, *J.F. Matthews s.n.* (NCU); Moore County, *B.A. Sorrie 9363* (GH, NCU).

OHIO: Athens County, *J.W. Thieret 56353** (KNK); Butler County, *M.A. Vincent 5693* (BHO, MICH, MO, MU, NY, OS, US, USF); Darke County, *M.A. Vincent 11001 et al.* (MU); Delaware County, *A.W. Cusick 35744* (MU); Franklin County, *A.W. Cusick 30637 & Shelton* (MU, OS); Hamilton County, *M.A. Vincent 7997* (MU, OS); Lawrence County, *A.W. Cusick 34753* (CM, MU, NY); Meigs County, *A.W. Cusick 35026* (MU); Muskingum County, *L.E. Brown 9235** (BRIT); Portage County, *A.W. Cusick 32216** (MU); Washington County, *A.W. Cusick 30632 & Ortt* (MU, OS).

OKLAHOMA: Bryan County, *C. Taylor 36151** (OKL); Carter County, *A. Buthod 4372 et al.* (OKL); Grady County, *L.K. Magrath s.n.* (OKL); McCurtain County, *C. Citty s.n.* (NLU).

OREGON: Benton County, *R.R. Halse 5718** (NY).

PENNSYLVANIA: Allegheny County, *S.A. Thompson 6354** (CM).

SOUTH CAROLINA: Florence County, *L. Swails s.n.* (USCH); Georgetown County, *J.B. Nelson 21469* (USCH); Greenville County, *N.E. Mullins 75318* (NLU); Lexington County, *J.B. Nelson 11950* (USCH); Newberry County, *C.N. Horn 4294* (USCH); Pickens County, *S.R. Hill 20097* (BRIT, GH, MO, NY, USF); Richland County, *J.B. Nelson 5737* (USCH).

TENNESSEE: Davidson County, *M. Guthrie 609* (TENN); Hamilton County, *J.T. Beck 3866* (TENN); Knox County, *A.W. Cusick 32241** (MU); Shelby County, *A. Evans s.n.* (MO, NLU, TENN).

TEXAS: Austin County, *M.H. Mayfield 1776 et al.* (BRIT, MO, TEX); Bexar County, *M.A. Vincent 4943* (ILL, MO, MU); Blanco County, *R.W. Sanders 5563* (BRIT); Brazoria County, *R.J. Fleetwood 11180** (SMU); Collin County, *S.R. Hill 4539** (GH); Dallas County, *B.L. Lipscomb 3386* (LSU, MU); Gillespie County, *R.W. Sanders 5339* (BRIT); Harris County, *L.E. Brown 8466* (NLU, SMU); Tarrant County, *B. Lipscomb 3472* (BRIT).

UTAH: Washington County, *L. Higgins 19902* (MO, NY).

VIRGINIA: Chesterfield County, *W.J. Hayden 3553* (NLU, URV); City of Lynchburg, *C. Leys s.n.* (MO, MU); Fairfax County, *T. Darling s.n.* (GMUF); Richmond County, *R.A.S. Wright 2417* (VPI).

WISCONSIN: Winnebago County, *M.A. Vincent 10805 & Lammers** (MU, OSH).

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Distributional Records of Selected Kentucky Fishes

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ABSTRACT

Distributional records for 14 species of fishes are included for Kentucky. *Phoxinus oreas* is recorded for the first time from Kentucky (Big Sandy River drainage, Pike Co.). Reported for the first time are drainage records for *Ichthyomyzon unicuspis* and *Clinostomus funduloides* (Licking River drainage, Bath/Rowan and Morgan cos., respectively); *Scaphirhynchus platorynchus* (Kentucky River drainage, Franklin and Henry/Owen cos.); *Cyprinella galactura* (Green River drainage, Barren Co.); *Notropis maculatus* (Tennessee River drainage, Graves Co.); and *Forbesichthys agassizii* (Tradewater River drainage, Caldwell Co. and Crooked Creek system, Crittenden Co.). Range extensions in the upper Cumberland River drainage for *Etheostoma crossopterum, E. nigrum,* and *E. percnurum* (Clinton, Pulaski, and McCreary cos., respectively) and for *Etheostoma chlorosoma* in the Green River drainage (Ohio Co.) are reported. The continued occurrence of the rare fishes *Acipenser fulvescens* (Ohio River, Lewis Co.), *Notropis maculatus* (Obion Creek system, Hickman Co.), *Noturus exilis* (lower Cumberland River drainage, Trigg Co.), and *Lota lota* (Ohio River, Bracken, Jefferson, and Livingston cos.) is noted.

INTRODUCTION

Kentucky has the fourth highest fish diversity in the United States, behind Alabama, Georgia, and Tennessee. Clay (1975) reported ca. 200 species occurring in the state; Burr and Warren (1986), 242 species. During the past 18 years the state total has increased with the resolution of several species complexes (Ceas and Page 1997; Page et al. 1992; Page et al. 2003; Wood et al. 2002). Continued monitoring of the distribution and status of the fish fauna is necessary for resource managers in species conservation planning and restoration efforts. In recent years, Burr et al. (1990), Warren et al. (1991), Cicerello and Laudermilk (1996), Ryon and Carrico (1998), and Eisenhour and Burr (2000) provided information on the status and distribution of several species. However, collections by university and state agency personnel, and records of species from sport and commercial fishing infrequently get reported outside of "gray" literature. Herein, the presence of one new species, substantial range extensions of several species, and current status information of some rare species for Kentucky is noted.

MATERIALS AND METHODS

All records reported were from collections made within the past 10 years by the authors or personnel from the Kentucky Division of Water (KDOW) or the Kentucky Department of Fish and Wildlife Resources (KDFWR), unless noted otherwise. Specimens were collected via seining or backpack electrofishing, unless noted otherwise. Preserved specimens were deposited at the Illinois Natural History Survey (INHS), Morehead State University (MOSU), or Southern Illinois University at Carbondale (SIUC). For specimens not vouchered in museum collections, the authors confirmed identification by personal examination of the specimens or examination of photographs. Common and scientific names for each species follow Nelson et al. (2004). Lengths are reported in standard length (SL) or total length (TL). Species accounts provide museum catalog number, number of specimens and their size range (in parentheses), stream name, receiving stream watershed (in parentheses), locality, county, and collection date.

RESULTS

Silver Lamprey

Ichthyomyzon unicuspis Hubbs and Trautman

MOSU 1588 (1; 111 mm TL), Licking River (Ohio River), in tailwaters below Cave Run Lake dam, Bath/Rowan cos., 23 Mar 2000.

Remarks: *Ichthyomyzon unicuspis* previously was unknown from the Licking River drainage. Burr and Warren (1986) reported this species as "often common" in the Ohio River but rare in streams of the remainder of the state. The specimen was a subadult attached to a muskellunge (*Esox masquinongy*) at the time of collection. The individual may indicate the presence of a reproducing population of *I. unicuspis* in the middle Licking River, or it may have been transported some distance by its host. Suitable spawning habitat appears to be present, although spring surveys in the Licking River by DJE revealed the presence of only *I. bdellium* (Ohio lamprey). *Ichthyomyzon fossor*, the "satellite species" of *I. unicuspis* (Hubbs and Trautman 1937), is a small-stream species not known from the Licking River drainage, so *Ichthyomyzon* ammoecetes from the Licking River mainstem with fewer than 53 myomeres are likely to be *I. unicuspis*.

Lake Sturgeon

Acipenser fulvescens Rafinesque

MOSU 1654 (1; 791 mm SL), Ohio River (Mississippi River), 60 m upstream of confluence with Kinniconick Creek, Lewis Co., 3 Jun 2000.

Remarks: *Acipenser fulvescens* was common in the upper Ohio River prior to 1915 but underwent a decline nearly to the point of extirpation by 1950 (Trautman 1981). The near absence of this species in collections from the middle part of the 20th century led Pearson and Pearson (1989) to consider it extirpated from the Ohio River. Outside of the Ohio and Mississippi rivers, vouchered records from Kentucky are rare; the only vouchered specimen we are aware of is a 1954 record (UL 7053) from the Cumberland River (Whitley Co.) (Burr and Warren 1986). Our record was an untagged specimen from the Ohio River caught by angling from the Kentucky bank. We are aware of three additional recent records from the Ohio River in Kentucky, including a tagged specimen, 118 cm TL and 12.1 kg, identified by D. Henley (KDFWR), from the Ohio River just above the confluence of the Wabash River, Union Co., Kentucky on 7 Mar 2000. The two other specimens, both verified by B. M. Burr (SIUC) from photographs, include one from south of Grand Chain, Pulaski Co., Illinois and Ballard Co., Kentucky, 1988 (Burr et al. 1990), and the other caught and released alive below Smithland Dam, Livingston Co., Kentucky, in April or May 1992 (B. Burr pers. comm. 2003). An additional specimen, ca. 120 cm TL and 18.5 kg, verified from photographs by DJE, was caught and released alive in late 2002 from the Mississippi River 4.8 km S of Columbus, Hickman Co., Kentucky.

It is possible that recent specimens represent a remnant Ohio River population, although they may have originated from Missouri or Indiana. *Acipenser fulvescens* has been stocked in the Mississippi and Missouri rivers in Missouri for 20 years (B. Fisher, Indiana Department of Natural Resources (IDNR), pers. comm., 7 Jan 2004), allowing a population to build in that state. Fisher has been studying what appears to be a native, reproducing population in the East Fork of White River in Indiana. This population, though small, appears stable and consists of diverse age classes. The tagged specimen collected from the mouth of the Wabash River originated from this population, suggesting that at least some of the specimens from the lower Ohio River may have migrated from Indiana. The juvenile (MOSU 1654) recently collected from the upper Ohio River is the first specimen collected from that part of the river in more than 60 years; its origin is unknown. Although these records document the continued presence of *A. fulvescens* in the Mississippi and Ohio rivers, it remains a very rare fish in the state and we recommend its Kentucky listing as endangered (KSNPC 2000) be continued.

Shovelnose Sturgeon

Scaphirhynchus platorynchus (Rafinesque)

Photo record (1; ca. 700 mm SL), Kentucky River (Ohio River), Pool 4 at Frankfort, RKM 104–105, Franklin Co., 10 May 2000; photo record (1; ca. 700 mm SL), Kentucky River (Ohio River), Pool 1, 1.6 km N of Eagle Creek mouth, RKM 16–17, Owen/Henry cos., 10 May 2000.

Remarks: All specimens were killed as a result of a large bourbon spill into the Kentucky River. Identifications of this distinctive species

were verified from photographs provided to DJE by W. Davis and K. Prather (KDFWR). Substantiated records in Kentucky are nearly absent outside of the Ohio and Mississippi rivers, although the presence of *S. platorynchus* in other large rivers of the state has been suspected (Burr and Warren 1986). Previous reports (Welter 1938) suggest it was once more common in these rivers, but anthropogenic changes have apparently reduced their populations. Locks and dams, common modifications of Kentucky's large rivers, contribute to sturgeon declines by preventing gene flow among populations, blocking migrations to critical spawning or feeding habitat, and altering flow regimens (van Winkle et al. 2002). The presence of *S. platorynchus* offers some hope that this species may also be present in other large, interior rivers of the state (e.g., Licking and Green), although it is likely to be rare, as it is in the Kentucky River.

Rosyside Dace

Clinostomus funduloides Girard

MOSU 1486 (1; 53 mm SL), North Fork Licking River (Licking River), at mouth of Bucket Branch, 3 km N of Leisure, Morgan Co., 19 Jul 1999.

Remarks: This is the first record of *C. funduloides* from the Licking River drainage. This species is native and common in the adjacent headwaters of the Little Sandy River (Burr and Warren 1986). Introduction by bait-bucket release seems likely because the specimen was taken at a fishing access and the close proximity (by road) to populations of *C. funduloides.* The closely related and morphologically similar redside dace, *Clinostomus elongatus,* is present in most of Bucket Branch, but we have not taken it in the lower 80 m of Bucket Branch or in adjacent reaches of North Fork Licking River.

Whitetail Shiner

Cyprinella galactura (Cope)

SIUC 33260 (5; 36–42 mm SL), Falling Timber Creek (Skaggs Creek), at KY 90, Barren Co., 17 Apr 1996; SIUC 51098 (3; 32–35 mm SL), Falling Timber Creek, at KY 90, Barren Co., 11 Feb 1998; SIUC 51094 (1; 34 mm SL), Falling Timber Creek, at KY 63, Barren Co., 17 Apr 1996; SIUC 51093 (3; 46–73 mm SL), Falling Timber Creek, at Glover Road ford, Barren Co., 10 Jul 2001.

Remarks: *Cyprinella galactura* inhabits the Big Sandy River drainage and the Cumberland River drainage from above the Cumberland Falls west to the Red River system (Burr and Warren 1986). The Big Sandy population is considered a probable introduction (Warren 1981; Burr and Warren 1986), but Powers and Ceas (2000) reported that it is generally distributed and abundant in Russell Fork and provided evidence in support of native status in the drainage. Our records are the first for *C. galactura* in the Green River drainage. The provenance of this species in the Green River is unclear but it could be the result of stream capture or bait-bucket introduction. *Cyprinella galactura* is one of several fish taxa (e.g., *Nocomis effusus* and *Notropis telescopus*) and a crayfish (*Cambarus cumberlandensis*) found in the upper Barren and Green rivers that typically inhabit the Cumberland River drainage (Warren and Cicerello 1983; Burr and Warren 1986; Lawson 2003). Falling Timber Creek is near areas identified as possible theaters of stream capture and faunal exchange between the upper Barren and Green rivers and Cumberland River tributaries (Lachner and Jenkins 1971; Warren and Cicerello 1983). The upper Barren and Green rivers are relatively well collected, and *C. galactura* apparently is restricted to Falling Timber Creek. A restricted distribution could result from bait-bucket introduction as well as stream capture. Additional research is required to resolve the status of *C. galactura* and other taxa shared by the Green and Cumberland rivers.

Taillight Shiner

Notropis maculatus (Hay)

MOSU 1979 (17, 43–51 mm SL), Little Joe Cr. (Obion Creek), along KY 307, 2.7 km S of Beulah, Hickman Co., 21 Jun 2003; SIUC 51097 (51, 22–31 mm SL), Three Ponds, (Obion Creek), 2.25 km SW of Hailwell, Hickman Co., 8 Sep 2003; MOSU 2195 (10, 45–60 mm SL), West Fork Clarks River (Tennessee River), old channel at KY 131, Graves Co., 16 Jul 2004.

Remarks: *Notropis maculatus* is a state-threatened species (KSNPC 2000) that primarily occupies undisturbed oxbow lakes,

swamps, and low-gradient streams. Prior Kentucky records are available only from these habitats in the Mississippi Alluvial Plain and Ohio River Bottomlands (Burr and Warren 1986). The new records expand the Kentucky distribution well into the Coastal Plain and represent the first records in the Obion Creek system since 1890 (Woolman 1892) and the first record in the Tennessee River drainage. It is somewhat surprising this species was not reported earlier from the adjacent Murphy's Pond area, considering that this area has been relatively well sampled and that *N. maculatus*, along with the state-endangered *Hybognathus hayi* (KSNPC 2000), were the two most common cyprinids at the site. In addition, prior collecting efforts in the Clarks River system did not reveal *N. maculatus* (Sisk 1969). Perhaps the difficulty of sampling the woody, debris-filled waters that *N. maculatus* typically occupies and the general rarity of the species explains why it has not been previously reported from these areas. The limited habitat unaffected by channelization in the Coastal Plain has not been thoroughly sampled and intensive collecting efforts may reveal *N. maculatus* to be more widely distributed.

Mountain Redbelly Dace

Phoxinus oreas (Cope)

SIUC 42429 (4; 32–54 mm SL), Abes Fork (Grassy Creek, Virginia), 0.1 km above Trace Fork, Pike Co., 7 Jul 1999.

Remarks: *Phoxinus oreas* is native to the central Atlantic slope from the York River south to Neuse River and to the upper New River in the Ohio River basin (Hocutt et al. 1986; Jenkins and Burkhead 1993). Because of its popularity with bait fishermen, *P. oreas* has been introduced into or is of questionable native status in additional Atlantic slope drainages and in the upper Tennessee River drainage. Two specimens collected by Powers and Ceas (2000) from Grassy Creek, a Russell Fork tributary in Buchanan Co., Virginia, represent the first Big Sandy River drainage record. Close proximity to a bait dealer and absence of this species elsewhere in the drainage suggest this record is a probable bait-bucket introduction (Powers and Ceas 2000). Our collection from Abes Fork, a 2–3 m wide headwater tributary to Grassy Creek, is the first Kentucky record. Additional specimens were observed in Trace Fork, a northern tributary to Grassy Creek in Kentucky, but were not retained. The presence of a brightly colored male and gravid females in Abes Fork suggests this is a reproducing and probably established population. As judged from Powers and Ceas (2000) and other recent collections in the Big Sandy drainage (e.g., KDOW, KSNPC), *P. oreas* apparently is localized in Russell Fork. However, additional collecting in headwater streams is needed to establish its distribution in the drainage.

Slender Madtom

Noturus exilis Nelson

SIUC 43166 (1; 79 mm SL), Donaldson Creek (Cumberland River), 0.5 km upstream of KY 164 bridge, Trigg Co., 19 Jun 2001.

Remarks: Donaldson Creek was one of the 10 localities reported by Burr and Warren (1986) for the state-endangered *Noturus exilis* (KSNPC 2000). Kornman (1995) found an additional locality in the South Fork Licking River (Pendleton Co.) but speculated that the specimens may be an undescribed form because of their unique morphology. Excluding Kornman (1995), the most recent collection of *N. exilis* was in 1983 from Kentucky Lake (Calloway Co.) and the species has not been reported from natural habitat since 1968 (Burr and Warren 1986).

Two specimens were collected on 19 Jun 2001 but occurred apart from each other. After examination, the second specimen was released, because the first specimen was already retained as the voucher (SIUC 43166). Both specimens were collected from cobble/gravel substrate in swift water. The vouchered specimen was a gravid female and the released specimen appeared to be male. The presence of a gravid female and a male is encouraging that the species still spawns in the state; however, *N. exilis* is among the rarest fishes in Kentucky and further efforts are needed to fully understand its current status in the state.

Spring Cavefish

Forbesichthys agassizii (Putnam)

SIUC 51051 (1; 66 mm SL), Piney Creek (Tradewater River), 0.15 km above an unnamed tributary, near Haile Road ford, Cald-

well Co., 16 Apr 2002. MOSU 2232 (1; 60 mm SL), Rush Creek (Crooked Creek), 0.4 km below US 60 and KY 641, Crittenden Co., 8 Jun 2004.

Remarks: These are the first records for *Forbesichthys agassizii* from the Tradewater River drainage and the Crooked Creek system (minor Ohio River tributary). Burr and Warren (1986) described *F. agassizii* as "sporadic to occasional and at times abundant" in the Cumberland, Green, and Tennessee river drainages within the Highland Rim and Shawnee Hills regions. As with other members of the family Amblyopsidae, *F. agassizii* is associated with cave systems and spring-fed streams (Burr and Warren 1986) within the karst subecoregions, Crawford-Mammoth Cave Uplands, Western Highland Rim, and Western Pennyroyal Karst Plain (Woods et al. 2002). Piney Creek and Rush Creek originate in the Crawford-Mammoth Cave Uplands subecoregion and the new records fill in distributional gaps within this area. The collection of single specimens from Piney Creek and Rush Creek may indicate the fish were recently flushed from a cave system, as they were collected in the spring.

Burbot

Lota lota (Linnaeus)

Photo record (1; ca. 575 mm TL), Ohio River (Mississippi River), at RM 915, near Birdsville, Livingston Co., 5 Jun 2002.

Remarks: In addition to the above record, which was verified by a photograph supplied to us by Paul Rister (KDFWR), two additional records from the Ohio River are from 1993. One was an ca. 610 mm TL specimen from RM 630, at West Point, Jefferson Co., Kentucky, identified by Benjy Kinman (KDFWR). The second specimen, ca. 600 mm TL, was from RM 443, below Meldahl Dam in Bracken Co., Kentucky. Identification of this specimen was confirmed from a photograph examined by LEK. *Lota lota* is listed as "special concern" in Kentucky (KSNPC 2000) due to its general rareness and the uncertainty of its status. This species infrequently has been taken in the Ohio River and other large rivers of the state (Burr and Warren 1986; Pearson and Pearson 1989). To our knowledge the 1993 and 2002 records are the only ones in the state since 1983. Older records include a specimen from Louisville (Jordan 1875), and eight specimens from the Ohio, Kentucky, and Licking rivers collected between 1953 and 1967 (Clay 1975). The origin of Kentucky specimens has been suggested to be escapees from stockings of private fishing lakes, waifs from more northern populations via the Missouri and Mississippi rivers, or a remnant, naturally reproducing population (Clay 1975; Trautman 1981). The 1875 record is prior to widespread stocking efforts, and we are unaware of any recent public stocking of *L. lota,* although private stocking is possible. We tentatively regard the origin of these recent specimens as from a native population but cannot determine whether they are waifs from northern populations or represent a remnant population in the Ohio River. Although reproduction of *L. lota* in the Ohio River basin has not been documented, dispersal from northern populations through numerous locks and dams to the upper Ohio River would be difficult.

Bluntnose Darter

Etheostoma chlorosoma (Hay)

SIUC 51069 (1; 37 mm SL), Sixes Creek (Indian Camp Creek), 0.5 km above Arnold-Baizetown Road, Ohio Co., 9 May 2002.

Remarks: *Etheostoma chlorosoma* is occasional to locally common in streams of the Coastal Plain and the Tradewater River drainage in the Shawnee Hills (Burr and Warren 1986). However, only two prior records are known from the lower Green River drainage, Caney Creek, Grayson Co. and Long Pond, Hopkins Co. (Retzer et al. 1983; Burr and Warren 1986). The persistence of the Long Pond population has been verified with the collection of several individuals in 1997, two specimens vouchered (SIUC 40064), and two specimens collected on 17 Aug 1999. The Caney Creek population represented the most upstream location until our collection from Sixes Creek, which extends the range ca. 105 km upstream in the Green River drainage.

Sixes Creek is a small, low-gradient tributary of Indian Camp Creek located in the Caseyville Hills subecoregion (Woods et al. 2002). The specimen was taken in swift water among gravel, silt, and clay substrate. KDOW personnel have conducted numerous stream surveys

in the lower Green River drainage and found no additional *E. chlorosoma* populations. The species may have been more common in the lower Green River drainage but extensive agricultural and mining practices probably have limited the species to a few relatively undisturbed streams in the Interior River Valley and Hills Ecoregion.

Fringed Darter

Etheostoma crossopterum Braasch and Mayden

SIUC 26374 (4; 41–59 mm SL), Spring Creek (Obey River), KY 127 bridge, Clinton Co., 21 May 1996; SIUC 51095 (9; 26–51 mm SL), Spring Creek (Obey River), 0.2 km upstream of KY 127, Clinton Co., 1 Nov 1999; INHS 57377 (15; 35–71 mm SL) Smith Creek (Spring Creek), Concord Church Road, 1 May 2000.

Remarks: *Etheostoma crossopterum,* a member of the subgenus *Catonotus, E. squamiceps* complex, is locally common in the lower Cumberland River in Kentucky (Burr and Warren 1986). Our records are the first for the species from the Eastern Highland Rim in Kentucky. These records, along with the record shown in Etnier and Starnes (1993), represent disjunct populations in the Obey River system. *Etheostoma crossopterum* is found predominantly in the Western Highland Rim and Nashville Basin physiographic provinces of Tennessee and Kentucky below the Caney Fork River system (Burr and Warren 1986; Etnier and Starnes 1993). Existing and disjunct populations of *E. crossopterum* are divided by a sister species, *E. olivaceum,* inhabiting the Caney Fork River system and adjacent tributaries of the Cumberland River. The two species are sympatric in the Cumberland River tributaries immediately downstream of the Caney Fork River confluence but are not syntopic (Etnier and Starnes 1993).

The geographically isolated *Etheostoma crossopterum* populations in the Obey River system are of possible interest to systematists. Members of the subgenus *Catonotus,* because of their fidelity to rocky headwater streams, exhibit considerable geographic variation and relatively high rates of speciation (Page and Schemske 1978; Page et al. 1992; Page et al. 2003). The SIUC (26374) and INHS (57377) collections had 1 and 10 breeding male specimens, respectively, which allowed us to distinguish them from other similar species. In addition, the specimens were taken from slab boulder/cobble habitat over fractured-bedrock in swift water.

Johnny Darter

Etheostoma nigrum Rafinesque

MOSU 1830 (1; 42 mm SL), Unnamed tributary to Fishing Creek (Fishing Creek), in Sulphur Springs Hollow, 0.7 km E of Cumberland Parkway Bridge over Fishing Creek, Pulaski Co., 23 May 2002.

Remarks: *Etheostoma nigrum* is widely distributed over much of Kentucky, but it is nearly absent from the Cumberland River drainage (Burr and Warren 1986). The closely related and endangered (KSNPC 2000) *Etheostoma susanae* (Cumberland darter) is present above Cumberland Falls and was formerly treated as a subspecies of *E. nigrum* (Strange 1998). Some specimens from Poor Fork of the Cumberland River (upper Cumberland River drainage) are morphologically intermediate between *E. nigrum* and *E. susanae* (Krotzer 1990) and apparently have an origin via stream capture from the headwaters of the Kentucky River (Strange 1998). Our collection of *E. nigrum* is only the second specimen collected from the middle Cumberland River drainage; the first (EKU 69) was collected in the Rockcastle River system (Starnes and Starnes 1979). Morphology of the Fishing Creek specimen is consistent with "typical" *E. nigrum.* Considering the limited Cumberland distribution of this headwater species, a stream capture origin for the middle Cumberland populations seems likely.

Duskytail Darter

Etheostoma percnurum Jenkins

SIUC 46940 (1; 41 mm SL), South Fork Cumberland River (Cumberland River), Blue Heron at canoe access, McCreary Co., 20 Sep 2000.

Remarks: *Etheostoma percnurum,* a federally endangered species (Biggins 1993), was recently reported from the South Fork Cum-

berland River in Kentucky (Eisenhour and Burr 2000). *Etheostoma percnurum* was found from the Tennessee border downstream ca. 8 km to Bear Creek with the most abundant populations (although still relatively small) located within a 3-km reach from Oil Well Branch to 1 km upstream of Troublesome Creek (Eisenhour and Burr 2000). The record reported herein extends the range ca. 8 km, from Bear Creek downstream to Blue Heron. However, below Oil Well Branch only two specimens have been collected. Within this area there is limited pool habitat that is relatively silt-free and the river below Blue Heron is impounded, which creates unsuitable habitat for *E. percnurum.*

The National Park Service in Big South Fork National River and Recreation Area protects the South Fork Cumberland River; however, the legacy of mining activities and other anthropogenic activities in the watershed continues to be a threat to the aquatic biota and stream integrity. For instance, Eisenhour and Burr (2000) observed "extremely turbid water" discharging from Bear Creek following a rain event, and McMurray and Schuster (2001) found that the aquatic macroinvertebrate and fish communities of Bear Creek remained severely impaired following initial reclamation efforts. Therefore, *E. percnurum* will most likely continue to remain rare below Oil Well Branch because of marginal habitat and historical anthropogenic activities.

DISCUSSION

Although large rivers have been severely altered by urban sprawl, mining operations, agricultural practices, and construction of dams, which affect the dispersal of its inhabitants, the collections of *Ichthyomyzon unicuspis, Acipenser fulvescens, Scaphirhynchus platorynchus,* and *Lota lota* indicate that monitoring of large rivers is necessary to update and define the range and conservation status of a species. In addition, monitoring of smaller systems is important in documenting new drainage records (e.g., *Phoxinus oreas* and *Forbesichthys agassizii*) and range expansions (e.g., *Etheostoma crossopterum* and *Etheostoma nigrum*) and in establishing data for long-term trend monitoring. Also, the number of records brought to our attention by commercial fishermen and recreational anglers underscores the importance of communication between these individuals and the scientific community. This information provides resource managers with current and credible data in their efforts to conserve and protect species and their aquatic habitats. This is especially important in Kentucky because the state does not have any state-endangered or threatened species laws. Therefore, protection of species, except for the federally endangered *Etheostoma percnurum,* is minimal.

ACKNOWLEDGMENTS

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Developing a Fast LDA Calculation to Model Group III Nitride Crystals

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ABSTRACT

A fast, semi-empirical computational method has been developed to model Group III-Nitride semi-conductor crystals. The calculation is based on an LDA approach with reduced range, local orbitals in the Harris approximation. Small tight-binding-like corrections are used to allow the calculation to produce quantitative lattice parameters and band gaps. This allows run-time to be substantially less than more rigorous LDA calculations. Preliminary results on small supercells are shown to agree well with the more rigorous calculations and experimental data.

INTRODUCTION

This work is focused on studying Group III Nitride (III-N) semiconducting crystals and their alloys using a fast Local Density Approximation (LDA) method. The nitrides have very wide band gaps and are very strong materials, which make ideal candidates for a wide range of device applications. These applications include ultraviolet (UV) and blue semiconductor lasers and detectors, high temperature and radiation resistance electronics, high frequency and low-noise communication devices, high-power radar and microwave devices.

Many of the structural and electronic properties of these III-N crystals have been well documented (Lambrect and Segall 1994; Morkoc et al. 1994; Schilfgaarde et al. 1997; Strite and Morkoc 1992). However, in part because the very short bond lengths of the nitrides make suitable substrates hard to find, these materials have been notoriously difficult to grow. Although techniques are improving all the time, crystals tend to be heavily n-type as grown. This phenomenon is usually attributed to nitrogen vacancies. Thus, p-doped nitrides are very difficult to make. In 1997, blue LEDs and laser diodes were achieved using GaN (Nakamura et al. 1997). The troublesome p-doping of GaN was done with Mg but required a very high concentration of the dopant to get acceptable hole concentration. One reason for this is that the acceptor level created by the Mg doping is quite deep. Another reason the high Mg concentration is necessary is that nitrogen vacancies and hydrogen impurities that are unintentionally present in CVD grown crystals compensate the Mg-acceptors. The acceptor states need to be reactivated after growth with either low-energy electron-beam irradiation or thermal annealing (Amano et al. 1989; Nakamura et al. 1992). The role of the H impurities in compensating the acceptor states has been explained by a careful analysis of the process using a rigorous LDA method (Neugebauer and Van de Walle 1996). Other dopants, like Cd, Hg, and Ca, have even deeper acceptor levels and would require even higher dopant concentrations.

The problem of p-doping tends to increase with the band gap as well. As the band gap widens both donor and acceptor levels tend to deepen, making doping more difficult. For example, it was found when GaN and AlN were alloyed ($Ga_xAl_{1-x}N$) the electrical receptivity in unintentionally doped samples increased rapidly as the Al concentration increased. This makes the development of UV LEDs and laser diodes much more difficult.

Clearly, LDA has made remarkable contributions in studying the energetics and defect structures involved with III-N semiconductors and their doping. However, alloying and doping require large supercells in order to get a meaningful result. The fully self-consistent LDA calculations are too time-consuming to be used in this respect. A more efficient method is needed to provide results for large-scale calculations.

The method in this work takes an LDA calculation based on local orbitals in the Harris approximation that has been recently developed at Auburn University, which has been able to accurately predict bond lengths and bulk moduli of many III-V and II-VI semicon-

ducting compounds in short times, including the pure III-N crystals (Dickerson 1997). Also the electronic structure calculated from this method has all the qualitative features of other, more rigorous, methods. Including a small correction to the *ab initio* LDA, allows the calculation to produce the basic quantitative bandstructure. The crystal structure is then fitted to the experimental lattice parameters with a simple addition of a small repulsive term to the total energy. With this correction, the model is applied to supercells to examine how the bandstructure changes with the addition of native defects.

This paper will discuss the LDA method used, the empirical correction employed to generate quantitative bandstructure, the results of this calculatation and the wide range of possibilities that can be actualized using this method.

COMPUTATIONAL METHOD

The object of designing the ab initio LDA calculation was to make it fast enough to handle supercells of 100 atoms or more. To do this a number of approximations needed to be made. In this section, the three most critical choices that were made to implement this fast calculation will be discussed.

The first choice was to use localized orbitals as our basis of calculation. Local orbitals are not orthogonal and there are no intrinsic parameters to control the convergence of the set as there are with plane waves. However, far fewer local orbitals are needed to produce converged results, which reduces run-time considerably. There has been considerable success for predicting the properties of III-V and II-VI semiconductor crystals using only one s-orbital and three p-orbitals per atom (Dickerson 1997). Local defects such as vacancies and impurities also produce local states that plane waves would struggle with. Also, local orbitals make the calculation more flexible. That is, they can be used in a wide variety of materials that are not fully periodic, e.g., single atoms, molecules, polymers, nanotubes, etc. Again, these are systems that are inherently difficult for plane waves to handle.

Specifically, minimal sets of compact orbitals we developed based on atomic orbitals. The atomic orbitals are determined by applying a fully self-consistent LDA calculation to the single atom problem using a robust gaussian basis set. The range of these atomic orbitals can then be reduced similar to the approach of Sankey and Niklewski (1989). Unlike Sankey, the orbital is not simply truncated; it is required that the orbital and its derivative remain continuous at all radii. The atomic orbital is fitted exactly inside a cutoff radius after which the orbital decays very quickly. In semiconducting crystals these compact orbitals produce converged results when interactions only up to third or fourth neighbor are included. In order to facilitate calculation these orbitals are expanded in a linear combination of gaussian functions and renormalized to produce a neutral atom. The orbitals used in this work are shown in Figure 1. The linear combination of Gaussian used to generate these functions are specified in Table 1, with A_i and α being the coefficients and decay constants of the expansion as determined in the equation:

$$\Phi_l(r) = \sum_i A_i \left(\frac{r}{2\alpha_i}\right)^l e^{-\alpha_i r^2} \qquad (1)$$

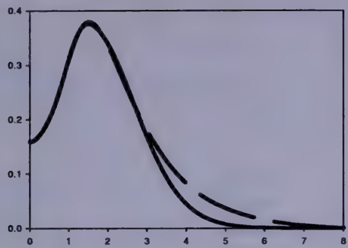
These radial functions are then multiplied by the real spherical harmonics to get the complete orbital.

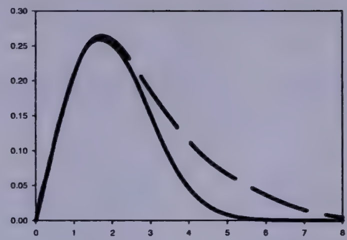
The second choice made to reduce runtime is the use of a pseudopotential so that the core electrons do not have to be treated explicitly. The pseudopotential is designed to reproduce the wave function and density of the full potential exactly outside a certain radius from the atom. This calculations uses the pseudopotential developed by Hamman, Schulter, and Chiang (1979). For GaN and InN it was necessary to treat the d electrons

→

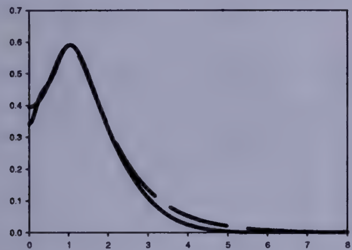
Figure 1. Orbitals used in LDA Calculation. The atomic orbital is the dashed line; the solid line is the reduced-range orbital used in this work. The reduced-range orbital is renormalized to produce a neutral atom. They are shown here unrenormalized to compare the shape and range of the reduced-range orbitals to the atomic orbitals. The horizontal axes are in Bohr radii (a_0). The vertical axes use units of $a_0^{-3/2}$ for the s orbital and $a_0^{-1/2}$ for the p orbital.

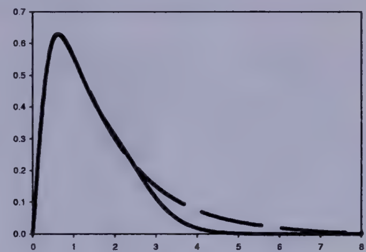
ALUMINUM **S** **P**



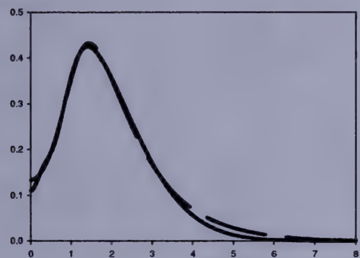


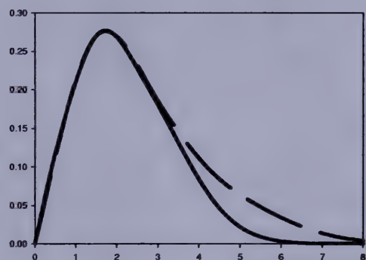
BORON



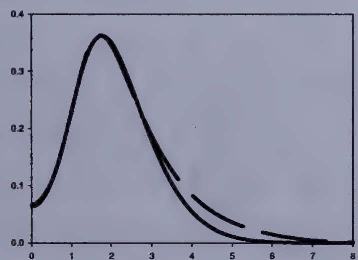


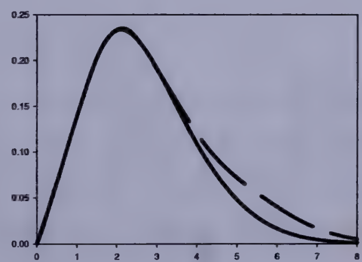
GALLIUM



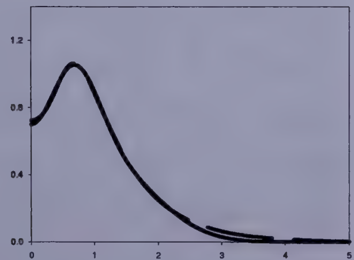


INDIUM





NITROGEN



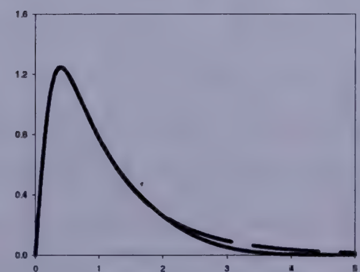


Table 1. Orbitals used for LDA Calculation. The coefficients and decay constants for atomic orbitals defined in equation (1) are listed below. The α_i's are in units of a_0^{-1} and A_i's are in units of $a_0^{-3/2}$.

	s		p		d	
	α_i	A_i	α_i	A_i	α_i	a_i
B						
i						
1	0.2115	0.7700	0.3105	0.7274		
2	0.6777	0.2615	0.6230	−0.5936		
3	1.9385	−0.9191	1.1540	1.2588		
4	5.4724	0.4757	2.1625	−0.0020		
5	15.3507	−0.2239	4.1663	0.7533		
N						
i						
1	0.5382	−4.3934	0.3917	0.5512		
2	0.8377	8.4704	0.8373	0.4601		
3	1.3250	−10.2842	1.9694	1.5532		
4	2.1410	6.3572	4.7858	2.0949		
5	3.5170	−0.9203	11.8171	1.4368		
Al						
i						
1	0.2020	−1.2501	0.2184	−0.5284		
2	0.4548	1.2943	0.4720	0.3872		
3	0.8910	−0.7715	0.9742	−0.4479		
4	1.6709	1.1736	1.9757	0.6019		
5	3.0719	−0.9092	3.9768	−0.7207		
6	5.5922	0.3445	7.9776	0.7320		
7	10.1273	−0.0532	15.9776	−0.4556		
Ga						
i						
1	0.1615	−0.7517	0.1916	0.6490	0.2198	−0.0378
2	0.5191	0.2330	0.3250	−1.1594	0.2801	0.1042
3	1.5350	0.6502	0.5170	1.7919	1.3568	2.0812
4	4.3605	−0.3837	0.8012	−1.5096	4.5796	17.8218
5	12.2633	0.1409	1.2234	0.5238	11.1092	35.6777
6					29.1552	−46.3553
In						
i						
1	0.1763	−0.9939	0.1145	0.1847	0.1617	−0.0058
2	0.3810	0.3646	0.2815	0.0557	0.5291	−0.3409
3	0.7856	0.9248	0.5738	−0.0496	1.4766	−2.7556
4	1.5898	−0.4106	1.0953	−0.1288	3.9508	−6.9840
5	3.1898	0.0475	2.0305	0.1580	10.6424	7.9237
6			3.7118	−0.1109	28.7114	−7.2654
7			6.7351	0.0405		

of Ga and In explicitly, since these states interact strongly with the deep 2s electrons in N. So a new pseudo-potential was developed using the same methods as Hamman et al. to handle these interactions. The new pseudo-potential, with the inclusion of the "semi-core" d-electrons, improved the prediction of the bond lengths from 7.4% error to 3.0% error in zb GaN with similar improvement for InN.

The third choice was to use the Harris Approximation (Harris 1985), which allows a number of different procedures to be implemented. The electron density of the crystal is chosen to be the linear combination of the atomic densities. This means that each atom remains neutral during the entire calculation, which, in turn, means the potential is short-ranged. The attractive core term and the repulsive Coulomb term cancel one another at large distances from the atom. These two potentials are combined and treated as a single potential, which is easily fitted to linear com-

Table 2. Equilibrium Crystal Structure of Nitrides using the fast LDA Method. Experimental results (Lambrect and Segall, 1994) are shown in parentheses. The lattice constant, a, is listed in Angstroms. Structural parameters c/a and u are the commonly defined unitless ratios for wz.

Compound	Crystal structure	a	c/a (wz only)	u (wz only)
BN	zb	3.653 (3.616)	—	—
	wz	2.575	1.649	0.3742
AIN	zb	4.198 (4.369)	—	—
	wz	2.985 (3.111)	1.600 (1.600)	0.382 (0.385)
GaN	zb	4.367 (4.501)	—	—
	wz	3.087 (3.180)	1.629 (1.624)	0.376 (0.375)
InN	zb	4.912 (4.97)	—	—
	wz	3.473 (3.533)	1.633 (1.611)	0.375 (0.375)

bination of gaussian functions. This allows for rapid, analytic evaluation of matrix elements, since the orbitals are also linear combinations of gaussians. Since this potential is linear in the density these matrix elements can then be stored in large arrays. The matrix element needed at run-time is then simply interpolated from this table.

Only the exchange-correlation term resists tabulation and analytic treatment due to the fact that it is not a linear function of density. To treat this term we fit the exchange-correlation potential to a linear combination of gaussian functions in the same manner as the Coulomb-core term. However, unlike the Coulomb term, the fit must be done at run-time. Due to the complicated form of the exchange, the fit is imperfect but results in a very reasonable approximation of this potential.

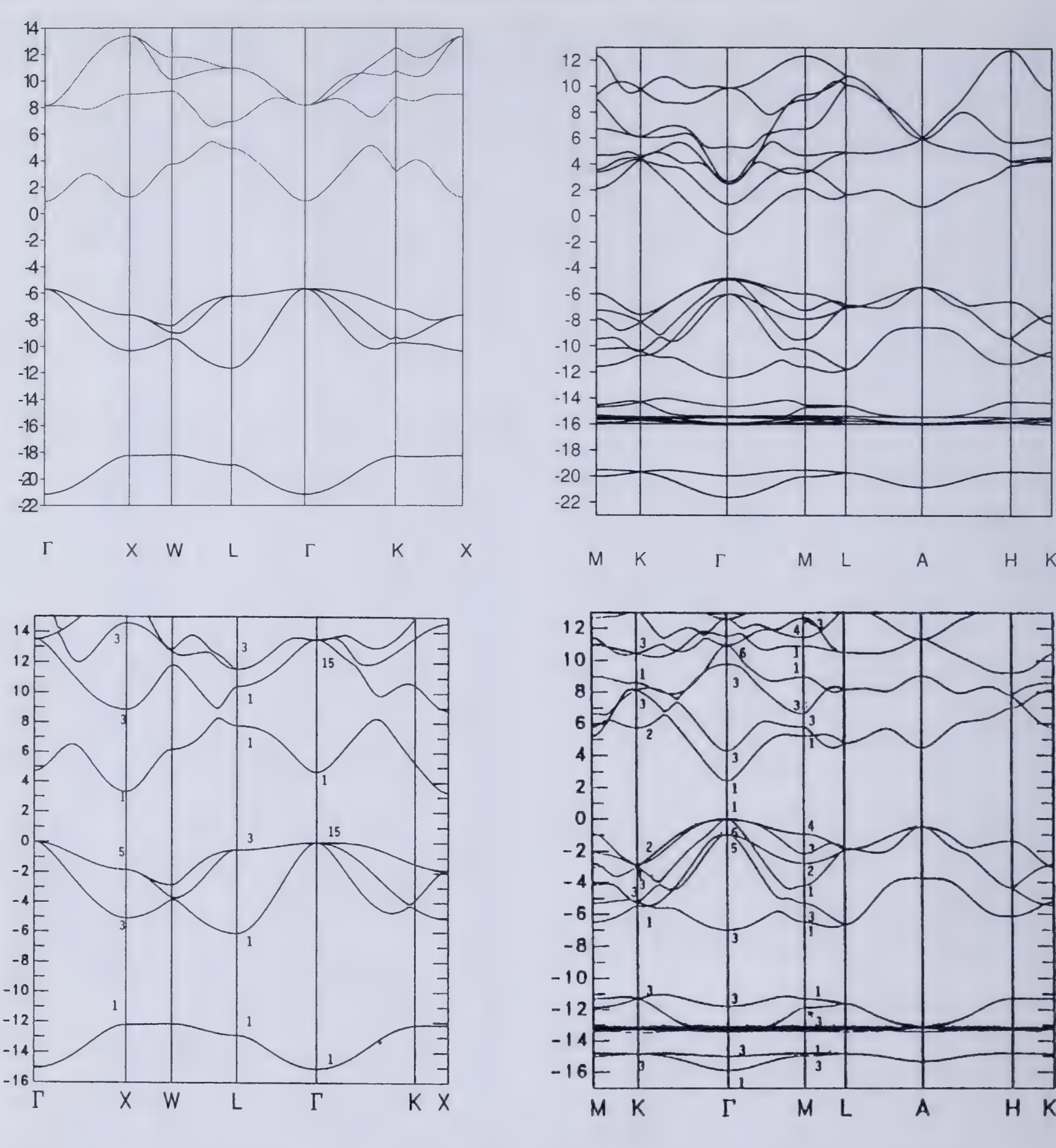
Using the local orbitals and the single calculation of the matrix elements in the Harris approximation, the calculation has the form of an ab initio tight-binding (TB) calculation. As such, it provides a useful way to conceptualize the interactions taking place in the crystal. The calculation has been used as the foundation of a semi-empirical model for the ground-state molecular structure and quantitative band structure for organic polymers. This model has shown itself to be a very durable and transferable method for calculating band gaps and bond lengths of many different types of conjugated, organic polymers (Yoder et al. 1999). This was possible because it has all the characteristics of a TB calculation but avoids the tedious fitting of a large number of parameters to experimental results that is found in purely empirical models.

This method has also proven itself to be a good method to produce structural properties of III-V and II-VI semiconductors. Bond lengths are generally within 2% of experimental values, and bulk moduli are within 10% (Dickerson 1997; Yoder et al. 1999). These results compare favorably to other slower, more rigorous LDA methods.

For the III-N crystals in this work, the results of this method using the orbitals defined by Table 1 are shown in Table 2. Also the band structure of the crystal can be extracted from the electron states produced by the calculation. The band structure produced by this calculation is qualitatively the same as the band structures that were calculated with a full-potential, self-consistent LMTO LDA calculation done by Lambrecht and Segall (Lambrecht and Segall 1994). This comparison is made in Figure 2 for zb-AlN and wz-GaN. Results for the other nitrides are similarly consistent with the more rigorous calculations.

EMPIRICAL CORRECTION

To make this method quantitative we have included small correction terms (much like a TB model) that we can fix to experimental band gaps and lattice parameters. Specifically, all that is needed to get the correct band gaps of the material is to shift the cation on-site s-states up to increase the size of the band gap at the experimental lattice parameters. Only in BN does the correction need to be applied to all the on-site B matrix elements, as the B s- and p-states are strongly intertwined for this crystal (Lambrect and Segall 1994). Also, the correction for BN reduces rather than increases the size of the band gap as the Harris LDA tends to calculate a band gap that is too large. The size of the corrections needed to produce the correct gap in each of these crystals is



(a) zb-AlN

(b) wz-GaN

Figure 2. Basic Band Structure of Nitrides. (a) The left column is the band structure of zinc-blend AlN calculated using the method of this work (top) and calculated using a full-potential, self-consistent LDA method (bottom) (Lambrect and Segall 1994). (b) The right column is the band structure of wurtzite GaN calculated using the method of this work (top) and calculated using a full-potential self-consistent LDA method [4] (bottom).

shown in Table 3. Since this correction is only added to states in the valence band, it affects the total energy only indirectly. To produce the measured lattice parameters of the material a small (~1 eV per bond at equilibrium bond length) exponentially decaying term is added to the total energy. The parameters of this scalar correction are listed in Table 3 as well, where A and α represent the coefficients and decay constants in the equation:

$$R(r) = Ae^{-ar} \qquad (2)$$

The final corrected results for these materials, both in the zb and wz crystal structures,

Table 3. Numerical Parameters used for an Empirical Correction to LDA Calculation.

Shift of on-site matrix elements		
Element	Shift (eV)	
B	−0.403 (p-states)	
Al	0.879 (s-states)	
Ga	1.256 (s-states)	
In	1.363 (s-states)	
Parameter for scalar energy term defined in equation (2).		
Bond	α (Å^{-1})	A (eV)
B-N	4.0	−114.3
Al-N	4.0	373.35
Ga-N	4.0	398.25
In-N	4.0	87.01

are listed in Table 4. Once we have corrected results for these materials we can apply this correct model to larger systems. We have applied these results to small supercells (64 atoms for zb, 72 for wz) just large enough to contain all third neighbors in the unit cell. An atom is then removed form the center of the supercell, and the first and second neighbors to the vacant site are allowed to relax. The first neighbors relax toward or away from the vacant site; the second neighbors relax toward or away from the first neighbors. When these sets of atoms relax, the band structure is recalculated. The supercell bandstructures are shown in Figure 3 for zb crystals with a N-vacancy and with then with a cation vacancy. The perfect crystal bandstructures (first column in Figure 3) are shown beside the vacancy bandstructures to illustrate how the electron states change. Since removing a nitrogen atom causes an odd number of electrons to be removed, one of the bands shown is half-filled and can be considered the donor or acceptor state. This half-filled bands are indicated with arrows on the figure. In all cases, we see that for the N-vacancy, the half-filled band is intertwined with some of the conduction bands. This indicates that it acts as a shallow donor. Although in the case of AlN several of the lowest conduction bands, including the half-filled band, have separated from the higher bands creating a small gap. Similarly, for the cation vacancy the half-filled band is the highest valence band and crosses the other filled bands. This suggests it acts as a shallow acceptor. These results are in good agreement with other experiments and with other calculations.

FUTURE WORK

We have begun establishing a clear, quantitative picture of the pure materials that will allow us to then add a wide range of defects and impurities to the materials and determine defect energies and at least the qualitative behavior of the donor/acceptor states these changes produce. The range of problems this method could then be applied to would be very large and could be used in the laboratory to test ideas of dopants, dopant concentrations, and other factors to guide their progress. Of particular interest is the p-doping of GaN, where a number of possibilities have been raised. It has been predicted that C on an N-site and Be on a Ga-site were better donors than the Mg dopant that is currently being used (Wang and Chen 2001). However, it has also been shown that Be energetically prefers an interstitial location over the Ga-site location (Van de Walle 1996). With our method, we should be able to confirm or contradict these findings and move toward finding a more efficient way to make p-type GaN. With the large supercells that this method can ad-

Table 4. Equilibrium Crystal Structure of Nitrides using the Modified LDA Method. Experimental results are shown in parentheses. The lattice constant, a, is listed in Angstroms. Structural parameters c/a and u are the commonly defined unitless ratios for wz. Band gap is listed in eV.

Compound	Crystal structure	a	c/a (wz only)	u (wz only)	Band gap
BN	zb	3.612 (3.616)	—	—	6.1 (6.1)
	wz	2.552	1.646	0.375	7.81
AlN	zb	4.369 (4.369)	—	—	6.03
	wz	3.106 (3.111)	1.601 (1.600)	0.383 (0.385)	6.20 (6.3)
GaN	zb	4.503 (4.501)	—	—	3.29 (3.3)
	wz	3.178 (3.180)	1.635 (1.624)	0.375 (0.375)	3.45 (3.5)
InN	zb	4.986 (4.97)	—	—	1.76
	wz	3.524 (3.533)	1.633 (1.611)	0.375 (0.375)	1.92 (1.9)

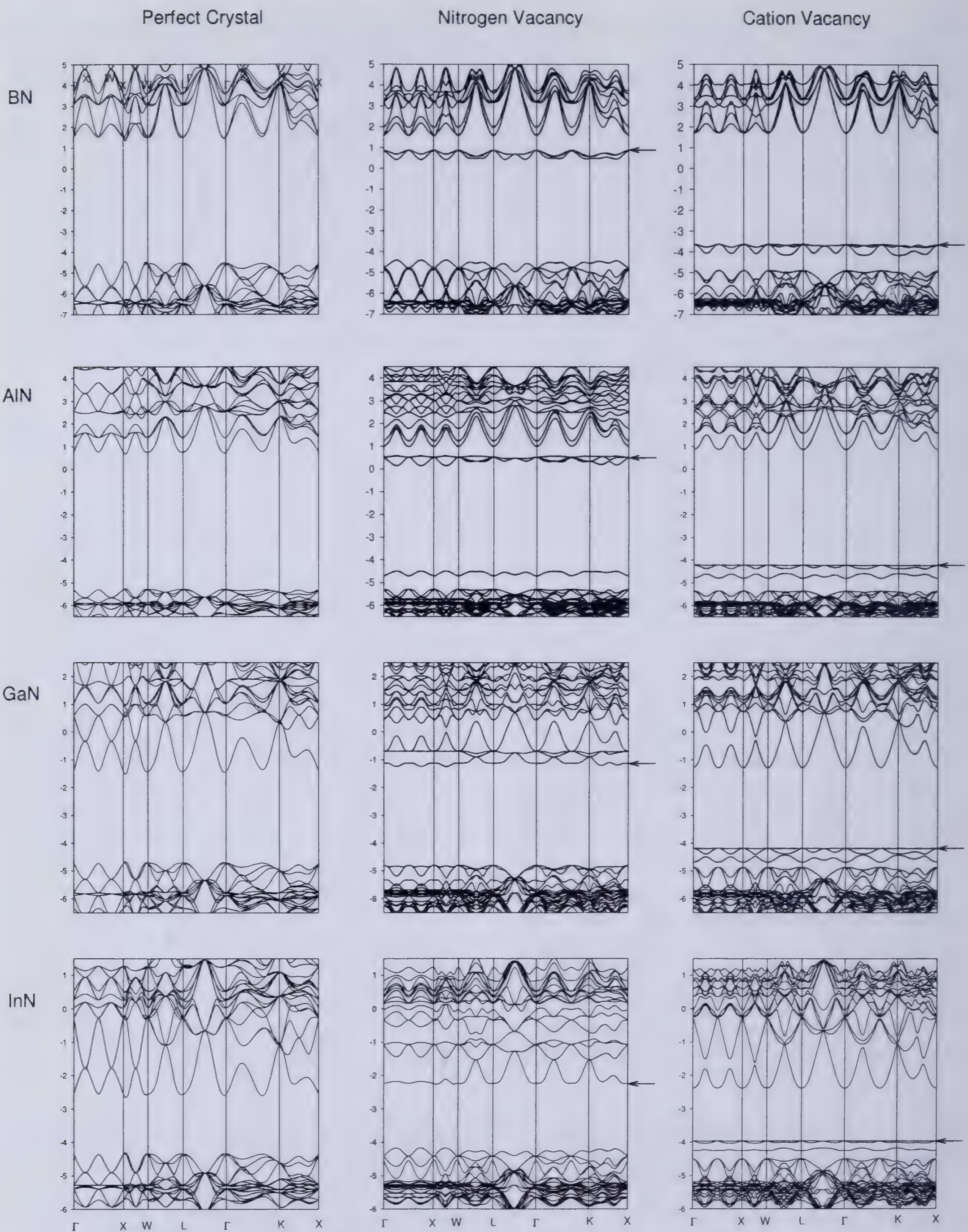


Figure 3. Supercell Bandstructure of Nitrides. Band structures produced with semi-empirical calculation for supercells of 64 atoms for the zinc-blend III-nitride crystals. The first column is the band structure of the pure crystal. The center column is the supercell with a single nitrogen vacancy and the right column is the supercell with a single cation vacancy. The arrows in the last two columns indicate the positions of the highest occupied (half-filled) bands.

dress, we can even perhaps model co-doping, calculate binding energy of donor-acceptor pairs, and study alloying of the materials in addition to doping.

Other potential applications include the investigation of the role of hydrogen in the doping process and modeling the dopant profile across interfaces in heterostructures. The role of hydrogen has already been shown to be a key player in Mg doping of GaN as discussed earlier. Oxygen impurities are found to produce "DX states" as they are allowed to relax in the crystal and are found in wurtzite lattices, but not in zinc-blende lattices (Chen and Sher 1995). Again the existence and stability of these states are straight-forward results of this calculation. The dopant profiles across interfaces in heterostructures which are necessary for almost all device applications and require large supercells, could also be studied. Understanding interfacial segregation is critical to controlling doping in these devices.

The potential application of this method is great and will contribute not only to practical device development but will also contribute to the fundamental understanding of basic semiconductor physics.

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Noteworthy Vascular Plants from Kentucky: A State Record, Range Extensions, and Various Species of Interest

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ABSTRACT

A total of 53 species is presented here, arranged in four groups: (1) a state record, *Polygonum densiflorum*, (2) range extensions in Kentucky (13 species), (3) various species of interest in the state (34 species, including 12 new populations for species listed and tracked by the Kentucky State Nature Preserves Commission), and (4) cultivated species possibly naturalized in the state (5 species).

INTRODUCTION

The species reported here primarily represent collections over the last several years based on student-related field research through the Berea College Herbarium (BEREA). Fourteen new state records involving BEREA student research were recently published (Abbott et al. 2001). Several Kentucky floristic projects involving BEREA students have also been published (Thompson et al. 1984, 1996, 2000; Thompson and FitzGerald Jr. 2003; Thompson and Fleming 2004; Thompson and Noe Jr. 2003) and others have appeared as abstracts (Abbott and Thompson 1993, 1994; Fleming et al. 1998; Thompson et al. 1995), but reports of most of the noteworthy species simply have not been formally published.

A recent dissertation (Medley 1993) and a recent book (Browne and Athey 1992) both provide lists of the vascular flora of Kentucky, but neither of them includes information from many of the specimens at BEREA. Ongoing research has also yielded additional records since the two checklists. Small regional herbaria such as BEREA are rarely utilized by workers outside the state; thus, their holdings remain largely unknown to other investigators.

There are several ongoing projects in the state that may culminate in a floristic atlas, a woody plant flora, and a manual of the flora of Kentucky. We report the following species to make knowledge of their presence available to other in-state workers and the botanical community at large. Medley (1993) was the primary source for information on the distribution of species in Kentucky, but a problem for us is that he occasionally cited a species' presence in a county based on knowledge of our collections without referring to our actual specimens. Nonetheless, with very few exceptions, we only report species here that fill in "gaps" in Medley's report. Browne and Athey (1992) was also referred to, but Medley has most of the same information, typically in greater detail. Any of the species below considered endangered, threatened, rare, or special concern by the Kentucky State Nature Preserves Commission (KSNPC 2000) are indicated after the name by KSNPC, status, and the year of the listing-report used. Gleason and Cronquist (1991) was used for distributional information outside Kentucky and is the source of our nomenclature. Data on taxa not present in Gleason and Cronquist were found in Radford et al. (1968). Unless otherwise indicated, all specimens are deposited at BEREA.

Fifty-three species are presented here, placed in four groups: (1) a state record, (2) range extensions in Kentucky (13 species), (3) various species of interest in the state (34 species, including 12 new populations for species listed and tracked by the Kentucky State Nature Preserves Commission), and (4) cultivated species that are possibly naturalized in the state (5 species).

KENTUCKY STATE RECORD

Polygonum densiflorum Meissner [Polygonaceae]

Native coastal plain species that ranges from New Jersey south to Florida, west to Texas, and interior to southern Missouri (Gleason and Cronquist 1991). It was to be expected in Kentucky (Beal and Thieret 1986), and it is present on the Mississippi Alluvial Coastal Plain in the contiguous states of Arkansas (Smith 1988), Missouri (Steyermark 1963), and Tennessee (Chester et al. 1997). Based on our examination of *Polygonum* specimens from Kentucky herbaria, Medley (1993) was correct in his assessment that there were not any Kentucky vouchers of *P. densiflorum.* Our collection is over 500 km east-northeast of any other collections from the above adjacent states.

Madison County: Berea College Forest, Red Lick Reservoir No. 2, ca. 2.2 km west of US 421 and KY at Bighill and 0.64 km south off gravel maintenance road; in cattail marsh near the earthern dam; infrequent. 24 Oct 2003; *Thompson 03-1208,* with J.R. Abbott.

RANGE EXTENSIONS

Castanea pumila (L.) P. Mill. var. *pumila* [Fagaceae] KSNPC Threatened (2000).

Johnson (1989) recorded this species as vouchered from eight counties in Kentucky but did not list Madison County. Medley (1993) stated there were five existing populations but that none was known with certainty to exist.

Madison County: Anglin Hollow, 2.5 km southeast on Long Branch Road from junction with Red Lick Road (KY 594); occasionally bush-hogged roadside wooded ledge at edge of field; rare, one shrub of several stems. 4 Oct 1993; *Abbott 6385,* with R.L. Thompson.

Crataegus coccinea L. [Rosaceae]

Medley (1993) cited only one specimen, from Letcher County without a collector or number. Ross Clark [EKY] first brought this species to our attention by identifying and annotating the following sheets (and others at BEREA and EKY).

Laurel County: Lily Surface-mined Area, 0.32 km south of Lily and 3.2 km east of Ky 25 off Lily-McHargue Road; rare, single tree on northwest outslope. 12 Jun 1981; *Thompson 541,* with D.D. Taylor.

Madison County: Berea College Forest, ca. 3.2 km east of Berea on KY 21; along old powerline right-of-way north of road. 22 May 1983; *D.D. Taylor 3473.*

Rockcastle County: John B. Stephenson Memorial Forest State Nature Preserve, Anglin Falls Ravine; south-southwest trending, dry midslope, rare. 28 Jul 1997; *Thompson 97-161,* with C.A. Fleming.

Geum laciniatum Murray [Rosaceae]

Previously known only along the Ohio River and in the coastal plain portion of western Kentucky (Medley 1993). As described in Campbell et al. (1994), the site below was discovered by Randy L. Mears in 1993.

Laurel County: London, 1.2 km south of junction with C.R. 1006 on U.S. 25, on west side of road; swampy bottomland remnant. 19 Jun 1994; *Abbott 6999,* with R.L. Mears. This site was re-visited on 8 Aug 2002, and it has been partially developed. A large area has been filled in and has several large gravel piles. There is still a small, open, swampy strip adjacent to the nearby swamp woods, but this species was not relocated.

Heracleum maximum Bartr. [= *H. lanatum* Michx.] [Apiaceae] KSNPC Endangered (2000).

A widespread circumboreal species. In Kentucky, known with certainty only from Harlan county in the southeast (Medley 1993).

Lewis County: Brush Creek Island in the Ohio River; late old-field; rare. 14 Jun 1995; *Gelis BC-254,* with R.L. Thompson.

Lathyrus hirsutus L. [Fabaceae]

Native to Europe. In Kentucky known only from seven western counties (Medley 1993).

Madison County: Berea College Campus, south of the Alumni Building and Athletic Field, adjacent to Scaffold Cane Road (KY 595); fallow field. 3 Jun 1999; *Abbott 12685.*

Ludwigia hirtella Raf. [Onagraceae]

Medley (1993) reported this species from Edmonson and Metcalfe counties, in addition to Pulaski county based on the population re-

ported here, which is vouchered only by our collection.

Pulaski County: near Woodstock, 2.4 km east on Ocala Road from junction with KY 39, north of Hazeldell Church of Christ; opening in seasonally wet upland woods, successional grass-sedge meadow, occasional. 15 Jul 1991; *Abbott 1016,* with R.L. Thompson.

Lycopodium appressum (Chapman) Lloyd & Underw. [Lycopodiaceae]
[= *Lycopodiella appressa* (Chapman) Cranfill] KSNPC Endangered (2000).

Known from Calloway county in the coastal plain portion of western Kentucky. Medley (1993) also cited the population listed here (based on our collection).

Pulaski County: near Woodstock, ca. 1.6 km east on Ocala Road from junction with KY 39, north of road along trail; small opening in seasonally wet upland woods; rare, only one population, fewer than 10 square meters. 5 Jul 1991; *Abbott 829,* with R.L. Thompson. The population, revisited in 1994, was in stable condition.

Ranunculus parviflorus L. [Ranunculaceae]

Previously known with certainty only from a few counties in western Kentucky (Medley 1993).

Laurel County: off Willie Green Road at Sinking Creek, 0.32 km downstream; fallow field north of creek. 10 May 1994; *Abbott 6797,* with R.L. Mears.

Madison County: Fort Boonesborough State Park; yard in campground; rare. 21 May 1994; *Abbott 6911.*

Ranunculus pusillus Poiret [Ranunculaceae]

Frequent in western Kentucky (Medley 1993).

Laurel County: London, 0.32 km east of junction with KY 80 on KY 192, then south of highway; in mowed wet meadow, along small seep. 10 May 1994; *Abbott 6801,* with R.L. Mears.

Ranunculus sardous Crantz [Ranunculaceae]

Native to Europe. Frequent in western Kentucky (Medley 1993). These collections show the species to be fairly well established in central and southeastern Kentucky.

Bell County: Fonde Surface-mined Demonstration Area; mixed hardwoods plantation, outslope; infrequent. 31 May 1989; *Thompson 89-949.*

Casey County: adjacent to the intersection of U.S. 127 and 70; in a wet meadow. 1 Jul 1988; *B. Hoagland 224* [BEREA; duplicate from EKY; identified as *R. pensylvanicus* in Hoagland and Jones (1992)].

Knox County: northeast of Corbin, ca. 2 km northwest of U.S. 25 on KY 830; grazed open roadside field; frequent. 25 Jul 1992; *Abbott 3344.*

Laurel County: North Corbin; east of U.S. 25W just north of Whitley County line; upper floodplain of Lynn Camp Creek. 2 May 1999; *Abbott 12554.*

Rockcastle County: east of Disputanta, 0.3 km east on Anglin Fork Road from Hammonds Fork Road, at junction with Anglin Creek and Clear Creek, wet open field; frequent. 9 May 1992; *Abbott 2090.*

Ranunculus sceleratus L. [Ranunculaceae]

Frequent along the Ohio River and in western Kentucky (Medley 1993).

Madison County: Fort Boonesborough State Park; sandy, open, upper beach along river; rare. 10 Jun 1992; *Abbott 2519.*

Urochloa platyphylla (Munro ex Wright) R. Webster [= Brachiaria platyphylla (Munro ex Griseb.) Nash] [Poaceae]

Native southeastern species (Radford et al. 1968). Previously known in Kentucky only from four western counties (Medley 1993).

Madison County: Fort Boonesborough State Park; river sand and mudflats; rare. 16 Aug 1992; *Abbott 4029,* with R.L. Thompson and R.L. Mears.

Veronica polita Fries [Scrophulariaceae]

Native to Eurasia. Reportedly rare in Campbell and Mason counties (Medley 1993). Medley (1993) reported *Veronica agrestis* (a very similar species) as frequent "probably throughout the state." We have never seen that species but have seen *V. polita* regularly enough to suspect that it is undoubtedly more common than collections indicate and that there may have been some confusion in Medley's report.

Boone County: south of Petersburg, 6.24 km north on KY 20 from junction with KY 18,

then northwest at junction with Woolper Creek; open field along lake on creek. 13 May 1994; *Abbott 6838,* with R.F.C. Naczi and R.L. Mears.

Garrard County: Camp Nelson Quarry, 2.72 km off KY 1845 from junction of U.S. 27 and 1.6 km west-northwest of Lamber Methodist Church; xeric aggregated gravel floor; infrequent. 1 Apr 1997; *Thompson 97-189,* with C.A. Fleming.

Jessamine County: waste place along Kentucky River, near Brooklyn Bridge. 30 Mar 1956; *D.M. Smith 1261.*

Madison County: Fort Boonesborough State Park; yards; frequent. 5 Mar 1992; *Abbott 1322,* with R.L. Thompson and G. Dandeneau.

VARIOUS SPECIES OF INTEREST

Achyranthes japonica (Miq.) Nakai [Amaranthaceae]

Native to eastern Asia. Previously reported for three counties along Tug Fork in eastern Kentucky. Medley (1993) also mentioned that the species is probably spreading, as confirmed by this collection and by Vincent and Cusick (1998), who reported it new to Ohio.

Lewis County: Brush Creek Island in the Ohio River; forested wetland; abundant. 3 Sep 1995; *Gelis BC-1077,* with R.L. Thompson.

Aconitum uncinatum L. [Ranunculaceae] KSNPC Threatened (2000).

Previously reported from five counties from northern to southeastern Kentucky, including Laurel (Medley 1993). This collection represents a new population.

Laurel County: London, University of Kentucky Feltner 4-H Camp; gravelly streambank; rare. 25 Sep 1999; *Thompson 99-1060,* with E.W.J. FitzGerald Jr.

Aegopodium podagraria L. [Apiaceae]

Native to Eurasia; escaped in northeastern United States. Previously reported only from Jefferson county in Kentucky (Medley 1993).

Lewis County: Manchester Island No. 1 in the Ohio River; mature bottomland hardwoods; rare. 8 Jun 1996; *Gelis M1-1211,* with C.L. Fleming.

Alopecurus pratensis L. [Poaceae]

Native to Eurasia, widespreadly naturalized in the United States. Previously reported as rare in a few counties in northern Kentucky (Medley 1993).

Madison County: Berea College Forest, 1 mi south of Bighill, 175 feet from KY 421 roadside on northwest trending side slope dominated by sericea lespedeza. 16 May 2003; *Thompson 03-50,* with D.B. Poindexter.

Cacalia suaveolens L. [Asteraceae]

Known mostly from western Kentucky but also from unverified reports in a few other scattered counties (Medley 1993).

Lewis County: Manchester Island No. 1 in the Ohio River; rare along shaded riverbank. 29 Jul 1995; *Gelis M1-767,* with R.L. Thompson, J.R. Abbott, and A.E. Shupe.

Camelina microcarpa Andrz. [Brassicaceae]

Native to the Old World. Previously known from a few counties in northern and central Kentucky (Medley 1993).

Laurel County: London, east of U.S. 25 on KY 1006 toward Levi Jackson Wilderness Road State Park, along gravel embankment of railroad tracks. 19 Jun 1994; *Abbott 5187,* with R.L. Mears.

Cardamine impatiens L. [Brassicaceae]

Native to Europe and introduced into the coastal states west to Michigan (Gleason and Cronquist 1991). Medley (1993) listed only Campbell and Jefferson counties in Kentucky.

Lewis County: Manchester Island No. 1 in the Ohio River; late oldfield; frequent. 4 May 1995; *Gelis M1-71,* with D. Snell.

Carex pedunculata Muhl. [Cyperaceae]

When the population below was vouchered, it was only the second known site for this species in Kentucky (Thompson et al. 2000). Now known in several counties in eastern Kentucky (Medley 1993).

Laurel County: Rock Creek Research Natural Area; mixed mesophytic hemlock forest near a small rockhouse recess on conglomerate ledge; very infrequent. 21 Apr 1985; *Thompson 85-77.* Verified by Dr. Robert F.C. Naczi, Delaware State University.

Chenopodium pumilio R. Br. [Chenopodiaceae]

Native to Australia. In Kentucky known previously from Trimble and Fayette counties (Medley 1993).

Lewis County: Manchester Island No. 1 in the Ohio River; seasonally flooded and eroded sandy bank, 28 Jul 1995; *Abbott 7808*, with R.L. Thompson and R.L. Gelis.

Chrysosplenium americanum Schwein. [Saxifragaceae] KSNPC Endangered (2000).

Rare in southeastern Kentucky (Medley 1993). Already known from Harlan County, but we report a new population.

Harlan County: Pine Mountain Settlement School; wooded spring seep into Issac's Creek; infrequent. 26 Mar 1998; *Thompson 98-14.*

Cypripedium calceolus L. var. *parviflorum* (Salisb.) Fern. [= *C. parviflorum* Salisb.] [Orchidaceae] KSNPC Threatened (2000).

Previously known from a few scattered locations in eastern Kentucky (Medley 1993). Our collection was the basis of Medley's (1993) report from Laurel county.

Harlan County: Sheppard Trail, near Pine Mountain Settlement School; on roadside embankment. 3 Jun 1997; *Thompson 97-1298.*

Laurel County: Rock Creek Research Natural Area uplands, northeast-facing pine-oak stand; rare. 7 May 1989; *Thompson 89-644.*

Disporum maculatum (Buckl.) Britt. [Liliaceae] KSNPC Special Concern (2000).

Known from several eastern counties in Kentucky, including Bell and Harlan (Medley 1993), but our collections voucher new populations.

Bell County: Fonde Surface-mined Demonstration Area; on highwall; infrequent. 21 Apr 1990, *Thompson 90-278.*

Harlan County: Pine Mountain Settlement School; north-trending mesic lower slope along the Split Rock Trail; infrequent to rare. 19 April 1988; *Thompson 88-279.*

Eriophorum virginicum L. [Cyperaceae]

Previously known in Kentucky only from Harlan county (Medley 1993).

Laurel County: south of Flatwoods and west of Frozen Camp Creek, in powerline right-of-way; wet sandy opening (disturbed drainage channel); several dozen fertile stems seen. 30 Jul 1993; *Abbott 5897,* with J.J.N. Campbell and S. Walker.

Floerkea proserpinacoides Willd. [Limnanthaceae]

Medley (1993) reported this species as rare near streams along the Ohio River in northern Kentucky, all west of these collections.

Lewis County: Manchester Island No. 2 in the Ohio River; mature bottomland hardwoods; occasional. 4 May 1995; *Gelis M2-75,* with D. Snell.

Gentiana flavida A. Gray [Gentianaceae] KSNPC Endangered (2001).

Previously reported from several scattered counties, some unvouchered or with uncertain identifications (Medley 1993).

Madison County: between Brushy Knob and Hacker Smith Mountain, near Lick Fork Creek; ca. 20 stems at edge of yard and remnant cedar glade thicket. 21 Aug 2002; *Thompson 02-392,* with M. Evans.

Geranium dissectum L. [Geraniaceae]

Native to Europe. Previously known from northern Kentucky in two counties, with a few other unverified reports (Medley 1993).

Madison County: Berea College Campus, south of Kettering Residence Hall and Agriculture Greenhouses; along edge of cultivated field adjacent to Scaffold Cane Road (KY 595); locally abundant. 23 Apr 1999; *Abbott 12458.*

Hexastylis heterophylla (Ashe) Small [Aristolochiaceae]

Known previously from Bell and Harlan counties (Medley 1993). Gleason and Cronquist (1991) treated the species as conspecific under *H. virginica.* According to Gaddy (1987), this species is distinct from *H. virginica.* Our plants have predominantly erect calyx lobes mostly 3–4 mm long (characteristics of *H. virginica*), and the details of the reticulation along the internal calyx of some flowers are also reminiscent of *H. virginica,* with relatively low relief and no distinct vertical ridges. Thus, we were tempted to follow Gleason and Cronquist. However, some calyx lobes are strongly reflexed and a few are ca. 5 mm long (especially when fresh), and some calices have a pronounced internal reticulation with distinct vertical ridges (characteristics of *H. het-*

erophylla). Even the flowers that are most like *H. virginica,* though, have anther connectives which are exerted beyond the anther (a distinctive *H. heterophylla* characteristic). Finally, Rob Naczi, of Delaware State University, who has field experience with many *Hexastylis* species, looked at our vouchers and then visited the site, concluding that, overall, our population is more like *H. heterophylla* than *H. virginica.*

Laurel County: ca. 100 m west of I-75 at junction with the Laurel River; a few plants near the river, more common upslope. 9 Apr 1994; *Abbott 6532.*

Linum usitatissimum L. [Linaceae]

Known previously from Fulton County in western Kentucky (Medley 1993) and reportedly vouchered from Madison County by Libby et al. (1997), although, contrary to the report, no specimen was left at BEREA.

Pulaski County: northeast of Hazeldell Church of Christ, ca. 1 mile west of junction with Alexander Road on Ocala Road; adventive in fallow field [near roadside edge]; rare. 2 Jul 1992; *Abbott 2847,* with R.L. Thompson.

Liparis loeselii (L.) L.C. Richard [Orchidaceae] KSNPC Threatened (2000).

Previously reported from a few counties in eastern Kentucky (Medley 1993). Thompson and MacGregor (1987) reported it from Bell County.

Clark County: abandoned limestone quarry, 1.4 km west of junction with KY 1924 on KY 418 and 0.32 km east of Lisletown (Halls on the River), adjacent to Kentucky River; raised spot in cattail-rush marsh; rare. 5 Aug 1994; *Thompson 94-701,* with E.W.J. FitzGerald, Jr.

Lobelia nuttallii Roemer & Schultes [Campanulaceae] KSNPC Threatened (2000).

Medley (1993) reported this species from four counties in southeastern Kentucky, including Laurel and Whitley, based partly on our collections. Campbell et al. (1994) reported several other populations in Laurel and Whitley counties, also based partly on our collections.

Laurel County: Lily Surface-mined Area, 0.32 km south of Lily and 3.2 km east of U.S. 25 off Lily-McHargue Road; old outslope pond embankment; rare. 28 Jun 1981; *Thompson 81-653,* with D.D. Taylor.

McCreary County: 0.8 km north off U.S. 27 from Scott County (TN) line, and west on Cline Road for 0.48 km; wet meadow portion of field adjacent to mixed hardwoods stand; infrequent. 10 Aug 1993; *Thompson 93-465.*

Whitley County: south of Bark Camp, 0.8 km south of F.S. 191 from junction with KY 1193; in a mowed powerline right-of-way along edge of mixed pine-oak woods; rare. 1 Aug 1993; *Thompson 93-447,* with J.R. Abbott and A.E. Shupe.

Lysimachia vulgaris L. [Primulaceae]

Native to Eurasia. Rare in three counties along the Ohio River, west of this collection (Medley 1993).

Lewis County: Manchester Island No. 1 in the Ohio River; unconsolidated shoreline; rare. 22 Jun 1996; *Gelis M1-1263,* with C.A. Fleming.

Oenothera linifolia Nutt. [Onagraceae] KSNPC Endangered (2000).

Rare in four western Kentucky counties and McCreary County in southeastern Kentucky (Medley 1993).

Pulaski County: near Woodstock, 2.2 km east on Ocala Road from junction with KY 39, NW of Hazedell Church of Christ; in fallow field; rare. 23 Jul 1992; *Abbott 3280,* with R.L. Thompson.

Papaver dubium L. [Papaveraceae]

Native to Europe. Only known from Campbell and Kenton counties in northern Kentucky (Luken and Thieret 1987).

Madison County: near Redhouse, Louisville and Nashville Railroad right-of-way, 0.32 km north on KY 388 from junction of KY 3372; infrequent. 17 May 1998; *Thompson 98-157.*

Nicholas County: between gas pipeline and roadside off U.S. 68, 4 km southwest of Ellisville; scattered group of 20 plants. 27 May 1995; *Gelis 326,* with R.L. Thompson and D. Snell.

Platanthera integrilabia (Correll) Luer [Orchidaceae] KSNPC Threatened (2000).

A federal candidate for listing (USFWS 1999). Medley (1993) listed three counties from southern Kentucky.

Laurel County: Marsh Branch Road (F.S. 774), ca. 0.96 km south of KY 192, then northeast ca. 0.48 km in ravine just north of F.S. 4108; wet swampy streamhead in open woods; one large extended narrow population of hundreds of plants. 31 Jul 1993; *Abbott 5954.*

Poa bulbosa L. [Poaceae]

Native to Europe. Known from one county in northern Kentucky and three counties in western Kentucky (Medley 1993).

Laurel County: London, Levi Jackson Wilderness Road State Park; near campground restrooms; forested roadside with mowed understory. 10 May 1994; *Abbott 6776,* with R.L. Mears.

Madison County: Berea, along slope behind buildings at southeast corner of Chestnut and Boone streets; remnant woodland slope. 20 Apr 1992; *Abbott 1911.*

Ranunculus ficaria L. [Ranunculaceae]

Native to Eurasia. Previously known in Kentucky from three counties along the Ohio River, west of these collections (Medley 1993).

Lewis County: Manchester Island No. 1 in the Ohio River; mature bottomland hardwoods; rare. 11 Apr 1995; *Gelis M1-35,* with R.L. Thompson.

Rhamnus frangula L. [Rhamnaceae]

Native to Eurasia. Previously only vouchered as naturalized in Jefferson and Laurel counties (Medley 1993). Our site represents a new population.

Laurel County: London, University of Kentucky Feltner 4-H Camp; mesic oak-pine forest below earthen dam embankment along creek; rare. 16 May 2002; *Thompson 02-44,* with E.W.J. FitzGerald, Jr.

Scirpus fluviatilis (Torr.) Gray [Cyperaceae] KSNPC Threatened (2000).

Previously listed for two counties in extreme western Kentucky (Medley 1993), and Jones (1994) reported it from Madison County. Our collection is the easternmost documented for Kentucky.

Bell County: Fonde Surface-mined Demonstration Area; settling pond edge; rare. 12 Aug 1989; *Thompson 89-1273.*

Sherardia arvensis L. [Rubiaceae]

Native to western Eurasia and northern Africa. Known previously from three scattered counties in Kentucky (Medley 1993).

Madison County: Fort Boonesborough State Park; mowed grass-legume field on west side of KY 388; locally abundant. 8 May 1996; *Abbott 8565,* with R.L. Thompson and B.S. Carlsward.

Sida hermaphrodita (L.) Rusby. [Malvaceae] KSNPC Special Concern (2000).

Medley (1993) reported this species from several other counties along the Ohio River in Kentucky.

Lewis County: Manchester Island No. 2 in the Ohio River; late oldfield; rare, one colony located near island head. 21 Jul 1995; *Gelis M2-517.*

Silene ovata Pursh [Caryophyllaceae] KSNPC Threatened (2000).

Previously reported from five counties (Medley 1993), including Bell, which is undoubtedly based on this voucher.

Bell County: Log Mountain Surface-mined Demonstration Area; wooded outslope; infrequent. 26 Jul 1985; *Thompson 85-1420,* with R.A. Straw.

Spiranthes lucida (H. Eaton) Ames [Orchidaceae] KSNPC Threatened (2000).

Medley (1993) reported this species from seven counties (but some as sight records only) in southern central to southeastern Kentucky.

Clark County: abandoned limestone quarry, 1.4 km west of junction with KY 1924 on KY 418 and 0.32 km east of Lisletown, adjacent to Kentucky River; rare, a single colony in the *Juniperus* xeric community at the edge of a small pond. 17 Jun 1994; *Thompson 94-426,* with J.R. Abbott.

Stellaria aquatica (L.) Scop. [= *Myosoton aquaticum* (L.) Moench] [Caryophyllaceae]

Native to Europe. Previously reported as rare on river banks and seeps of five counties along the Ohio River (Medley 1993).

Lewis County: Brush Creek Island in the Ohio River; unconsolidated shoreline; frequent. 27 May 1995; *Gelis BC-184,* with R.L. Thompson.

Vallisneria americana Michx. [Hydrocharitaceae]

Known previously from two counties farther west (than this collection) along the Ohio River and from a few other scattered counties in Kentucky (Medley 1993).

Lewis County: Manchester Island No. 2 in the Ohio River; aquatic bed; infrequent. 17 Aug 1995; *Gelis M2-860,* with R.L. Thompson.

CULTIVATED SPECIES POSSIBLY NATURALIZED

The following species are included as they appeared to us to have been non-cultivated. It is possible, however, that they were planted many decades ago, although no homesite remnants were seen. Some of them were certainly spreading in the immediate area. Nonetheless, if it were possible to know the entire site history, perhaps all but the *Euonymus* and *Ligustrum* could be seen as having spread from cultivation or as persisting and may not truly be naturalized (e.g., Nesom 2000).

Akebia quinata (Houtt.) Dcne. [Lardizabalaceae]

Native to eastern Asia. Previously known in Kentucky from Jefferson County (Medley 1993).

Rockcastle County: Daniel Boone National Forest, ca. 0.64 km from KY 1004 on a forest service road; roadside pine clear-cut area; rare. 27 Jul 1991; *Thompson 91-739,* with D.D. Taylor.

Euonymus alata (Thunb.) Siebold [Celastraceae]

Native to eastern Asia. Medley (1993) reported this species as infrequently naturalized in a few scattered counties in Kentucky.

Lewis County: Manchester Island No. 1 in the Ohio River; oldfield; rare. 8 Jun 1996; *Gelis M1-1207,* with C.A. Fleming.

Madison County: Berea College Campus, south of the Alumni Building and Athletic Field, along cross-country trail past Brushy Creek; mostly disturbed secondary hardwood forest, but with several scattered, exotic, usually-cultivated plants; apparently spontaneous, dozens of individuals seen along creek. 3 Jun 1999; *Abbott 12681.*

Kerria japonica (L.) DC. [Rosaceae]

Native to China and Japan. Medley (1993) mentioned it from cultivation. Gleason and Cronquist (2001) included this species as an occasional escape from cultivation.

Harlan County: Pine Mountain Settlement School; persisting in mixed mesophytic woods from site of old burned library near Issac's Creek; rare. 19 Apr 1988; *Thompson 88-219.* This species was also collected at Pine Mountain Settlement School as an apparently cultivated shrub near a woodland margin far from any buildings (*Abbott 8585*). This latter collection demonstrates how a planting in an out-of-the-way place could later be seen as possibly naturalized, especially when decades have passed and site usage has changed.

Madison County: Berea College Forest, ca. 3.68 km east of Berea on KY 21 from junction with KY 595; pine-oak woodland strip along north side of road; one multi-stemmed shrub growing at base of, and surrounded by, native trees and shrubs. 23 April 1993; *Abbott 4674.* Perhaps persisting from cultivation long ago but there is no evidence or local record of an old homesite in the immediate area. Not apparently spreading here. This could represent an escape from recent cultivation somewhere nearby.

Ligustrum obtusifolium Siebold & Zucc. [Oleaceae]

Native to eastern Asia. In disturbed areas in Fayette and Oldham counties (Medley 1993). Based on several specimens recently annotated at BEREA by Ross Clark (EKY), this species is much more commonly naturalized than previously reported.

Garrard County: Camp Nelson Quarry, 2.7 km on KY 1845 from junction with US 27; dry quarry floor near highwall talus area; rare, two fruiting shrubs. 2 Aug 2004; *Thompson 04-1105.*

Madison County: Berea College Campus, south of the Alumni Building and Athletic Field along cross-country trail past Brushy Creek; mostly disturbed secondary hardwood forest, but with several scattered, exotic, usually-cultivated plants; numerous individuals of various sizes present. 3 Jun 1999; *Abbott 12682.*

Rockcastle County: John B. Stephenson

Memorial Forest State Nature Preserve, Anglin Falls Ravine; northwest-trending lower slope in *Pinus-Liriodendron-Quercus* community; infrequent. 3 Oct 1997; *Thompson 97-344*, with A.N. Allen.

Lycium barbarum L. [Solanaceae]

Native to temperate Eurasia. Documented as sparingly naturalized along a few roadsides and creeks in four Kentucky counties, mostly on limestone-based soils (Medley 1993).

Clark County: roadside ditch along KY 418 ca. 0.16 km from junction with KY 1924; rare, site discovered by Ross Clark. 26 Sep 2003; *Thompson 03-1066*.

Madison County: Berea College Campus near Agriculture Annex; clustered in cut-back *Morus alba*. 19 Sep 1985; *Thompson 85-1632*.

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A PCR-Based F_1 Hybrid Screen Using the Beta-actin Genes from the Sunfishes *Lepomis cyanellus* and *L. macrochirus* (Centrarchidae)

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ABSTRACT

Hybrids between closely related fish species are widely propagated in aquaculture and known to occur in many instances under natural conditions. The resulting hybrids often do not have characters that distinguish them definitively from the multiple possible parent species, making field identification difficult or impossible. This can be particularly difficult with juvenile specimens and adults who are not displaying breeding coloration. *Lepomis* species are capable of producing many hybrids, and *L. macrochirus* × *L. cyanellus* hybrids are produced commercially and released into private and public waters. This hybrid, easily distinguishable by morphology from its parent species, provided a good model to test a molecular identification tool. In this study I describe a method to design an unambiguous polymerase chain reaction (PCR) screen for hybrids that can be performed on living or dead specimens, requires minimal and non-invasive tissue sampling, is rapid and inexpensive, and can be adapted for any species.

INTRODUCTION

The problem of correctly identifying hybrid offspring in closely related fish species is compounded by the complex genetic interactions that may occur between the chromosomal contributions of the parent species. Morphological traits can often identify candidate parental species but confirmation is seldom possible. Moreover, F_1 hybrid offspring may vary from each other due to crossing over in parental gametes, and the outcome may also differ between crosses if the gender of the particular species is varied (i.e., male *Lepomis cyanellus* × female *L. macrochirus* versus female *L. cyanellus* × male *L. macrochirus*) (Childers 1967). Even non-hybrid juveniles of some species can be difficult to identify correctly without tedious scale counts and dissection. Application of a molecular test with unambiguous results provides a definitive method to ascertain correctly the identity of a fish as well as to determine whether or not the specimen is a hybrid.

Common molecular techniques for fish identification are allozymes, microsatellite markers, or other DNA profiling strategies such as restriction fragment length polymorphisms (RFLPs) (Avise and Saunders 1984; Neff 2001). While these are effective and reliable in most circumstances, characterizing polymorphisms in the genome can be time-consuming, and evaluating the methods for statistical reliability can be overwhelming and costly. With relatively inexpensive reagents for polymerase chain reaction (PCR) and increasing knowledge of fish genomics, more precise strategies are possible.

An alternative approach, presented here, is to look for intronic polymorphisms in a conserved gene that can distinguish species that are otherwise nearly identical at the genetic level. This approach has several advantages. First, consensus primers to exonic sequences can be used to isolate the gene from multiple species for sequence analysis. Second, polymorphisms can be chosen that are not hypervariable in nature. Therefore, these polymorphisms are likely to be found universally within a single species with few exceptions. Third, because introns tend to span large distances, it may be possible to design primer sets that can screen for multiple species with multiple possible PCR product lengths in a single reaction. Lastly, because the length will likely be much longer than a typical microsatellite, analysis can be performed on simple agarose gels instead of high resolution polyacrylamide gels.

The gene chosen in this study is the cytoplasmic beta-actin gene, a member of the actin gene family. This gene is highly conserved across kingdoms, has little or no variation at the amino acid level, and is highly conserved at the nucleotide level in teleosts. There are at least six, and possibly as many as nine, actin family members in teleosts (Venkatesh et al. 1996). Beta-actin can be distinguished from

other actins by the signature amino acids at each terminus of the coding sequence. Within the beta-actin gene, the introns are valuable phylogenetic markers (Lee and Gye 2001). In this study, primers were designed to hybridize to unique regions in the first intron of the beta-actin gene in two sunfish (family Centrarchidae), creating different products in each species and providing a rapid technique for identification of hybrids. The application of this approach to other species and other families is discussed.

MATERIALS AND METHODS

Specimens

Lepomis cyanellus and *L. macrochirus* specimens were collected from South Elkhorn Creek in Woodford County, Kentucky, and from Town Branch in Fayette County, Kentucky. *Lepomis cyanellus* × *L. macrochirus* hybrid specimens were purchased from the Jones Fish Hatchery (Cincinnati, OH).

Isolation of Genomic DNA

Small fin clip biopsies (5 mm^2) from eight fish were incubated in 250 μl lysis buffer (100 mM Tris, 5 mM EDTA, 0.2% SDS, 200 mM NaCl), and 5 μl proteinase K (from a 100 μg/ml stock solution) at 52°C for 2 hours. Samples were vortexed and centrifuged in a microfuge for 8 minutes at top speed. The supernatant was then transferred to a fresh tube containing 400 μl isopropanol. The tube was inverted 10 times and centrifuged for 1 minute at top speed, and the supernatant was removed and discarded. The pellet was washed with 95% ethanol, dried briefly, then resuspended in water.

Cloning of Beta-actin Genes

Beta-actin whole gene primers were designed to hybridize with the first 9 and the last 9 codons of the cytoplasmic beta-actin gene based on conserved sequences in available database entries. Whole gene primers were as follows: Forward: 5′ atg gat gat gaa atc gcc gca ctg gtt 3′; Reverse: 5′ tta gaa gca ttt acg gtg gac gat gga 3′. Cycle parameters were: 95°C for 15 minutes, followed by 30 cycles of (95°C for 30 sec, 47°C for 30 sec, 72°C for 1 minute). The genomic fragment produced from each fish was ca. 2 kb and was cloned into the pGEM-T Easy vector (Promega). Candidate clones were sequenced and compared to known beta-actin genes for verification, and the exons were determined using consensus splice sites and the virtually invariant beta-actin amino acid sequence.

Amplification of Polymorphic Regions

The polymerase chain reaction was carried out with each sample using the following amounts: 5 μl genomic DNA (from a 10 ng/μl stock), 1.5 μl of each primer (from a 10 ng/μl stock), 12.5 μl of 2× Thermoscript PCR master mix (Marsh Bioproducts), and water up to 25 μl. The Exon 1 Forward Primer (EX1F) sequence is: 5′ tgg ttg ttg aca acg gat ccg gta tgt gca 3′. The *L. macrochirus* reverse primer (MRP) is 5′ tta aaa ggt aaa gat ctt gac tac atg tac g 3′. The *L. cyanellus* reverse primer (CRP) is 5′ tgg tta gac ctc att aga tgt cag cat atg 3′. The cycle parameters for the primers used in this study were as follows: 95°C for 15 minutes, followed by 30 cycles of (95°C for 30 sec, 47°C for 30 sec, and 72°C for 1 minute). Samples were electrophoresed on a 1.5% agarose gel and visualized using ethidium bromide staining.

RESULTS

Exon 1 and intron 1 of the beta-actin genes from *L. cyanellus* and *L. macrochirus* are shown in Figure 1. The first 27 nucleotides of the gene were included in the synthesized primers used to isolate the gene and as such do not necessarily represent the true genomic sequence and are not included here in calculation of identity. All data discussed is based on codons 10–366 out of the 375 total codons. The total intronic sequence between the start and stop codons is 49 nucleotides longer in *L. cyanellus* than *L. macrochirus*, but it is nearly identical otherwise (Figure 2). The majority of the differences observed reflect gaps or insertions and occur within the first intron. The sequence identity for the total sequence is 94.6%. The presumed amino acid sequence has over 95% identity with published protein sequences from *Oncorhynchus mykiss* (Johnson 2002) and *Cyprinus carpio* (Liu et al. 1990). Variation in the coding regions between *L. macrochirus* and *L. cyanellus* is confined to the third position of the codon in most instances, as expected (data not shown).

Primers were designed to exploit the differ-

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                              Exon 1 forward primer ——►
L.m. ATGGATGATGAAATCGCCGCACTGGTTGTTGACAACGGATCCGGTATGT
L.c. ATGGATGATGAAATTGCCGCACTGGTTGTTGACAACGGATCCGGTATGT

GCAAAGCCGGTTTCGCCGGAGACGACGCCCCTCGTGCTGTCTTCCCCTCCATCG
GCAAAGCCGGTTTCGCCGGAGACGACGCCCCTCGTGCTGTCTTCCCCTCCATCG

TTGGTCGCCCCAGGCATCAGgtgagtgattgatcgccagcacaataaagccaca
TTGGTCGCCCCAGGCATCAGgtgagtgattgatcgccagcacaataaagccaca

ccgtttttta-----------------tggattttaaaacacatttactatcc
ctggttttaataagaacttgctgattatggattttaatacacatttactgacc

taattacactcctaagcaattaaaatta----ttcctgaatttcttgattgtta
taattacactcctaaacaattaaaattaattattcctgaatttcttgattgtta

aatgaaaattgctttgct--------------aatgaggtctaaccactaagc
gctgaaaattgctttgctcatatgctgacatctaatgaggtctaaccactaagc
                  ◄——— L. cyanellus reverse primer
aacatttacatgcgcaacctgattaaataagtactatattatgggaaatattcc
aacatttacatgcacaacctaattaaa----tactgcattataggaaatattcc
                 ◄——— L. macrochirus reverse primer
ctcattgtatt----tgacacacgtacatgtagtcaagatctttacctttaat
cacatggtattgccgagacacaaatacatgtagtcaagatcttaacctttt-aat

taggaactgctacatatccatgttttgttttt-------aacaagtttgtcttg
taggaactgctacatagcaatgttctcttttttttctttaacaagtttgtattg

tttgtcatgtcctgttcagGGAGTGATGGTGGGTATGGGCCAGAAGGACAGCTA
tttgtcatgtcttgttcagGGAGTGATGGTGGGTATGGGCCAGAAGGACAGCTA
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Figure 1. The genomic sequence of the beta-actin gene in *Lepomis macrochirus* and *L. cyanellus.* The exonic sequence is in capital letters, intron 1 is in lowercase letters. All of exon 1 and the beginning of exon 2 are shown. The locations of primers are italicized. Gaps are indicated by dashes in the sequence.

ences observed in the first intron, particularly where gaps were present. PCR amplification with the primers indicated in Figure 1 produced species-specific products when assayed by gel electrophoresis. Figure 3a shows the results when the Exon 1 Forward Primer (EX1F) was paired with the *L. macrochirus* Reverse Primer (MRP). There are 355 base-pair long bands present with *L. macrochirus* DNA (lanes 2–4), absent with *L. cyanellus*

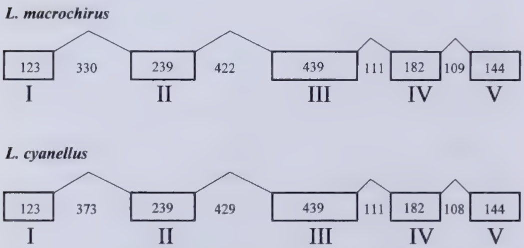

Figure 2. Gene structure of the beta-actin gene in *Lepomis macrochirus* and *L. cyanellus.* The nucleotide lengths of exons I through V are indicated in boxes and the lengths of introns are indicated between the boxes. The gene contains four introns between the start and stop codons. 5′ and 3′ untranslated regions are not included here.

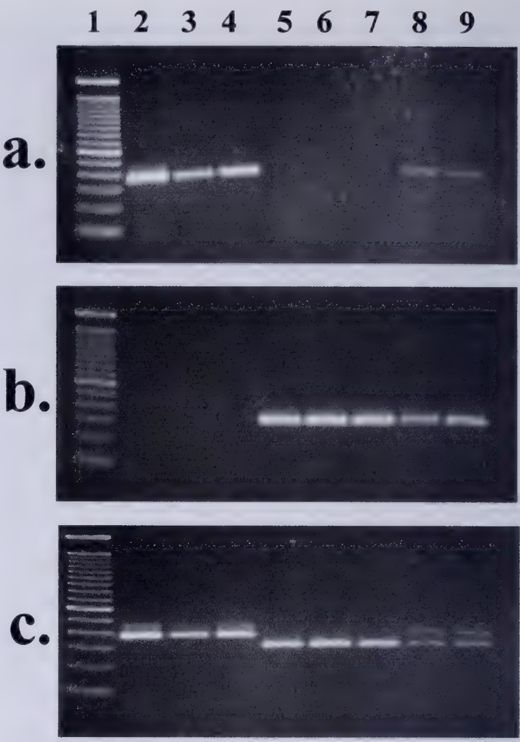

Figure 3. PCR products are species-specific. Panel 3a shows the results when the "Exon 1 Forward Primer" is paired with the *Lepomis macrochirus* Reverse Primer (MRP). The expected product is 355 basepairs long. Bands are only present with *L. macrochirus* DNA (lanes 2–4) and hybrid DNA (lanes 8–9). Panel 3b shows the results when EX1F is paired with the the *L. cyanellus* Reverse Primer (CRP). The expected product is 282 basepairs long. Bands are only present with *L. cyanellus* DNA (lanes 5–7) and hybrid DNA (lanes 8–9). Panel 3c shows the products of a reaction using EX1F, MRP and CRP. Both species exhibit their expected size bands (lanes 2–4 and 5–7), and the hybrid DNA produces both 282 and 355 basepair products.

DNA (lanes 5–7) and present with genomic DNA from the hybrid specimens (lanes 8–9). Figure 3b shows the results when EX1F was paired with the the *L. cyanellus* Reverse Primer (CRP). There are 282 basepair long bands absent with *L. macrochirus* DNA (lanes 2–4), present with *L. cyanellus* DNA (lanes 5–7) and present with genomic DNA from the hybrid specimens (lanes 8–9).

The products of a reaction using EX1F and both reverse primers simultaneously (MRP and CRP) are shown in Figure 3c. In this scenario, each single species is limited to produc-

ing the specific band corresponding to its chromosomal sequences, but the hybrid samples allow amplification of both the 282 and 355 basepair products. It may be pointed out that a potential technical limitation is that competition for the common primer in each reaction (in this case, the forward primer) may cause unequal intensity in the resulting bands when both templates are present, as in the hybrid. Most likely this could be overcome by tedious selection and optimization of primer length and GC content, but the unequal intensity does not interfere with the interpretation and is considered unimportant in the final result. The fact that both bands are present *only* if both parental versions of beta-actin are present is of primary interest here. This limitation was not a factor in this primer combination, but to normalize intensity between single species genomic DNA samples and hybrid DNA samples (which contain half of the template amount for each product), 30% more hybrid product was loaded into the gel for analysis.

DISCUSSION

In this study, I describe a technique for developing a rapid and unambiguous test for identification of F_1 hybrid fish. This technique is not suitable for determining introgression, gene flow, or F_2 crosses. Instead, it should be used as a simple identification tool and can be rapidly adapted to any species. For example, in 10 days or less the sequence data for the beta-actin gene can be acquired from a fin biopsy, and species-specific primers can be synthesized. Even though the intronic primers used here, by design, have limited application outside of *L. cyanellus* and *L. macrochirus*, the consensus primers for the start and stop codons have potential for use in any species, and could be even more adaptable if the wobble codon positions were made degenerate in the primer design. Preliminary data indicate that these primers will not be restricted to the centrarchids. At this time, I have already isolated the beta-actin gene from five esocids using these primers and the techniques described herein. Therefore, there is little doubt that this technique could have broad application.

This is also the first description of a *Lepomis* beta-actin genomic sequence. The predicted gene structure appears to have conserved exon lengths with other teleosts as distantly related as *Takifugu rubripes* (Venkatesh et al. 1996) and *Esox masquinongy* (author's observation). The amino acid sequence is highly conserved, and it is noteworthy to mention that beta-actin is a widely used phylogenetic marker that could have potential for characterizing the relationships between the Centrarchids as well as other families (Baldauf et al. 2000; Bhattacharya and Weber 1997; Goodson and Hawse 2002). The implications of this will be discussed elsewhere when a complete collection of *Lepomis* sequences is obtained.

ACKNOWLEDGMENTS

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Growth of Stygobitic (*Orconectes australis packardi*) and Epigean (*Orconectes cristavarius*) Crayfishes Maintained in Laboratory Conditions

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ABSTRACT

This study reports on maintenance and growth of the cave crayfish, *Orconectes australis packardi*, and the epigean crayfish, *Orconectes cristavarius*, within laboratory conditions for 1 and 2 years. The *O. a. packardi* survived well compared to the *O. cristavarius* in captivity. The poor survival of the epigean species was probably due to unsuitable conditions. The epigean as well as the cave crayfish molted and grew in captivity, but without any significant difference in molt frequency between species. In the first year, total body length was obtained to assay growth, whereas in the second year the more accurate measure of post-orbital carapace length was used. The ability of *O. a. packardi* to adjust to captivity is likely due to their lower metabolic rate and ability to handle hypoxic stress better than epigean species.

INTRODUCTION

The growth and maintenance of cave crayfishes in laboratory conditions is not well documented but advantageous to know for several reasons. Although field studies may inform us of those aspects of growth that occur in nature, they are complicated by many variables that cannot be controlled. For instance, with respect to crustaceans as well as mammals, periodic climate changes over a yearly cycle or over several years can bias data obtained during a brief period of time. This is also relevant for cave crustaceans that are influenced by surface streams varying seasonally in temperature. Resources, such as food and shelter, also may impact one subset of the population but not another (e.g., location dependent), as is known to occur in sand crabs (Siegel and Wenner 1985). Standardization of such variables in controlled laboratory studies allows them to be assessed and tested for integration in field studies. In addition, knowledge of how well stygobitic (i.e., aquatic cave obligate) animals survive in a holding facility is of use in case of a need to temporarily circumvent species eradication by acute environmental impacts. Such disturbances occur with land development, producing a high sedimentation and anthropogenic pollutants known to be lethal to crustaceans in general (Fingerman 1985).

Growth measurements of stygobitic crayfishes in the field have proven to be a difficult task, and this is likely the reason for the scarcity of quantitative data. Problems of marking and recapture of cave crayfish species over the molt cycle have been addressed by Cooper (1975), Cooper and Cooper (1976), Hobbs III (1978), and Weingartner (1977). Using various marking approaches, individuals have been recaptured and identified for 5 years in one study (Cooper 1975), 3.5 years by Weingartner (1977), and 2 years in another study (Hobbs III 1973). Recapture studies of troglophilic crayfish (*Cambarus laevis* Faxon) over a year and ones maintained in jars held within surface and cave streams were conducted by Weingartner (1977).

Field monitoring of crustacean development in a variety of karsts with wide-ranging dynamics is necessary since water temperature, environmental space, and food resources are not constant within all karst systems. Surface stream runoff from summer to winter alters water temperature in caves. For example, karst waters in an Indiana cave varied from 11.6 to 8.0°C after a rain on a snow-laden surface (Poulson 1964). In the second longest cave in Kentucky (Coral Cave, 38.46 km of passages) and the third longest (Sloans Valley Cave, 37.70 km of passages), both in Pulaski County, stygobitic crayfish (*Orconectes australis packardii* Rhoades, 1944), occur within a short distance of a karst window (30 m in Coral Cave) and are exposed to fluctuating water temperatures. In contrast, the longest cave

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in Kentucky (Mammoth Cave, Edmonson County, 580 km of passages) contains deep bodies of water with little variation in temperature as well as regions more directly influenced by surface stream temperatures (Barr and Kuehne 1971; Packard 1888; Poulson 1992). Since water temperature is the major environmental trigger for inducing molting in cave crayfishes (Jegla 1966, 1969), one would expect crayfish populations within caves to have varied molt cycles. Thus, growth rates of crayfishes may vary depending on the region of a cave in which the animals are being monitored. Estimates of age and developmental rates of cave crayfishes suggest that they develop very slowly and that some can live for long periods (~30 years) (Cooper 1975; Cooper and Cooper 1978; Weingartner 1977), but direct measurements over this long have not been made. In addition, since temperature is a regulating environmental factor in crustacean development, generalizations for all cave crayfishes are impractical since the animals inhabit various physiomorphic regions of caves.

Here we report on a preliminary study to monitor the growth and laboratory maintenance of a stygobitic species (*Orconectes australis packardi* Rhoades, 1944) and an epigean (surface-dwelling) species (*Orconectes cristavarius* Taylor, 2000). We used both juvenile and mature crayfish so we could determine if various age groups would survive in controlled conditions. In this preliminary study, we report on the laboratory conditions used and the growth of these species maintained over a 1-year and in some cases a 2-year period in a defined laboratory setting.

MATERIALS AND METHODS

Orconectes a. packardi were collected in the Sloans Valley Cave System at the "Appalachian Trail" in a pool of water measuring 3 m wide, 6 m long, and at most 0.3 m deep. *Orconectes cristavarius* were obtained from a relatively fast-moving surface stream (Fourmile Creek) by Fourmile Road in Clark County, KY. Species identification was confirmed by Dr. Gunther Schuster of Eastern Kentucky University for *O. cristavarius* and by the taxonomic key provided in Hobbs Jr. et al. (1977) for *O. a. packardi.*

The crayfishes were transported to the laboratory in Lexington, KY, in water obtained from their environment. They were then transferred to individual aquaria (33 × 28 × 23 cm; water depth 10–15 cm) and held as isolates throughout the study. Some individuals were housed successfully for a year. Containers were cleaned biweekly and animals were fed with commercial fish food pellets (Aquadine), which is marketed as "shrimp and plankton sticks: sinking mini sticks." Since this consists of ground-up fish it would appear to be a suitable and nutritious diet for crayfishes. Fragments of cleaned chicken egg shell also were placed in the containers as a source of calcium. The chloramines Lexington uses for water purification were removed by carbon-based filters for the aquaria water. The carbon-filtered water was held in a 190 liter (50 gallon) plastic tank and aerated for several days before utilization. Bacteria and algae were allowed to grow in the tank in order to detoxify any NH_4^+ and convert it to nitrite and nitrate, since NH_4^+ is known to be toxic to crustaceans (McRae 1999). Cave animals were maintained in total darkness except for feeding, cleaning, and measuring. Epigean animals were exposed to a low light level with a light cycle of 16:8 (light : dark) produced by full spectra lights (General Electric). When the aquaria were cleaned or when measurements were obtained, observations were made if the animals had molted by the appearance of the animal and by the presence, in the holding tank, of the chelae from the old exoskeleton. Neither species would fully consume the chelae after they consumed the rest of the old exoskeleton. The temperature was maintained at 16 to 17°C throughout the year; this was the temperature of the laboratory. At one period the laboratory temperature was uncontrolled as mentioned in the results during the second year of this study. Aquaria for both the surface and cave species were stored in the same room over the same period of time, and the tanks containing the cave species were covered with black plastic to block light. Aquaria were marked with numbers so that individuals could be monitored.

Total body length (tip of rostrum to the end of telson) was measured to an accuracy of 1 millimeter with a flexible plastic ruler. Measurements were obtained four times during the first year. After the first year we learned that the total body length measure is likely in-

accurate because of the flexibility of the jointed abdomen. So for a subsequent year of holding some of the same crayfishes in captivity, along with the addition of new individuals, the postorbital carapace length (from the posterior, dorsal surface of the orbital cup to the end of the carapace directly posterior to the eye cup) was used. These measures were made with calipers (Swiss Precision, Switzerland, 0.1 mm). As for the first-year study, four different time points were used throughout the year. A similar periodic sampling had previously been used by Weingartner (1977). The percent growth was determined by: [(postmolt length − premolt length)/(premolt length)] × 100.

RESULTS

The epigean and stygobitic species grew and molted in captivity, but the survival rate over the first and second years was lower for the epigean species than for the stygobite. The highest mortality for the epigean species occurred after 7 months, although two of them died after 4 months. The stygobitic species demonstrated a better survival rate, with only one dying after 4 months and another after 9 months. The one that died at 4 months appeared to do so during ecdysis since the exuvium was still attached to the body. The individuals that died are represented in Figure 1 as line plots that do not fully extend to the end of 1 or 2 years. The lines terminate at the period when measurements were last obtained.

The frequency of molting was substantially higher (9 of 12 animals) for the epigean species within the first few months of containment. A second molt was noted for only one of the epigean species within the first 12 months (Figure 1B, note two asterisks). Of the four surface crayfish that progressed to the second year of study only the largest one died. Three of the 13 stygobites molted during the same first few months and two of them after 9 months. The individuals that molted are indicated with asterisks within the period of time a molt was noted (Figure 1). Only in the stygobitic species was it observed that some individuals actually had a reduction, instead of an increase, in their body length after a molt. This phenomenon has also been reported for *O. a. australis* by Cooper (1975), and in an earlier report Creaser (1934) stated that in the

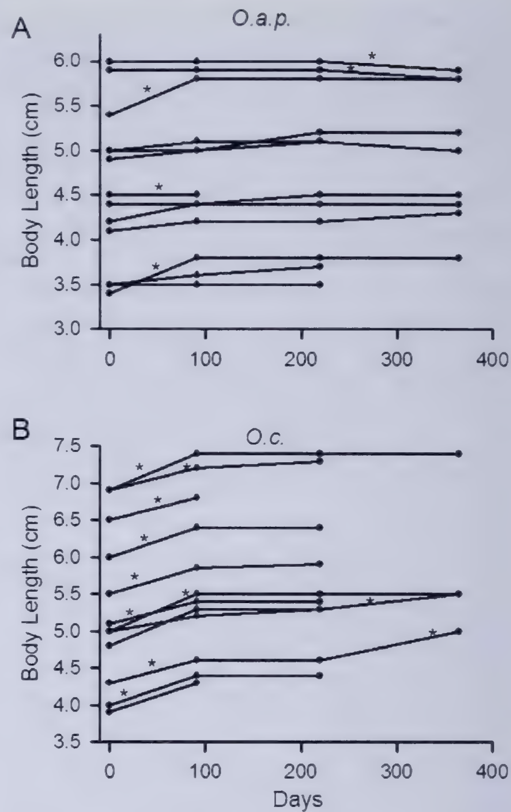

Figure 1. Growth of stygobitic (*Orconectes australis packardi*) and epigean (*Orconectes cristavarius*) crayfishes in laboratory conditions. Growth curves were obtained by measures of total body length of the animals maintained as isolates (A) *Orconectes australis packardi* (*O.a.p.*) and (B) *Orconectes cristavarius* (*O.c.*) for a 1-year period in captivity. Asterisks denote that a molt occurred in that time period. Individuals that died have their growth plot terminated at the last date a measurement was taken. There was substantial mortality for the epigean *O.c.* species after 7 months of containment. Initially N = 13 for *O.a.p.* and N = 12 for *O.c.*

crayfish *O. propinquus* (Creaser used the name *Faxonius propinquus*) growth was not always associated with a molt when the male changed in sexual form. We are fairly confident in the recorded dates of molts for each animal; however, there were a few animals, in both species, that indicated a growth in body length without a molt being observed. This lack in changes of length we account to not being able to fully stretch the abdomen of the animal consistently during all measures. Perhaps the muscles between the segments of the abdomen were relaxed more during some

measures than others. This problem was avoided during the second year of this study in which the postorbital carapace length was measured.

Four freshly caught stygobites were added to the study for the second year because we released a few from the first-year study back in the cave since we clipped one pair of antennules from some individuals at the end of the first-year study to aid in another study in antennule growth within a molt cycle. Figure 2A contains the data obtained during the second year of the study of the stygobites. The ones in the graph depicted with circles were from the first-year study and the ones represented by triangles were crayfish newly added to the study. The results obtained in the second year indicate that the animals did increase in length during a molt although a minor amount in some cases. For the stygobites, three out of the four added to the study in the second year molted within the first 2 months. Six out of the eight molted within the last 150 days, which was likely a result of the laboratory having environmental temperature swings for a period of about 2 weeks with temperatures reaching as high as 28°C.

The few epigean crayfish appeared to increase in size to a greater degree than the stygobitic species. As in Figure 2A, the lines in Figure 2B depicted with circles were ones from the first-year study, and the crayfish represented by triangles were newly added epigean species. Only three animals were carried over from the first-year study. One died within 1 month, another within 3 months, and the last one within 7 months of the second study in which postorbital carapace length was measured. As in the first-year study, the newly added epigean crayfish also showed a low resistance to laboratory rearing since a good number had died before the year was completed.

To determine the extent of the difference in growth between the two species during the first year of the study, a percent change for each individual was determined and the means of the percent differences were compared between species (Figure $3A_1$). Since so many animals died in the epigean population in the period between 220 days and 365 days, the 220-day measurement session was used to calculate the percent difference in growth for this species during the first year. Most of the growth for the epigean species occurred earlier than that for the stygobitic species. As mentioned earlier, the total body length was likely subject to error, but since such measures of surface crayfish species are rapidly made for aquaculture purposes, we retained these data since they can be of use. The growth measures during the second year for the stygobitic and epigean crayfishes of the postorbital carapace

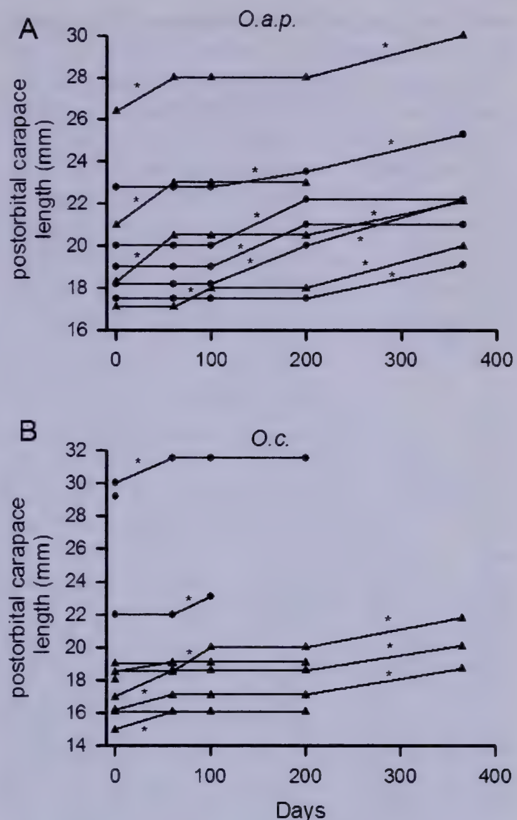

Figure 2. Growth curves of stygobitic (*Orconectes australis packardi*) and epigean (*Orconectes cristavarius*) crayfishes as measured by postorbital carapace length. Plots for *Orconectes australis packardi* (*O.a.p.*) (A) and *Orconectes cristavarius* (*O.c.*) (B) are shown. Animals held throughout a second year in captivity (solid circles) as compared to 1 year (solid triangles) are indicated. Asterisks denote that a molt occurred in that time period. The individuals that died have their growth plot terminated at the last date a measurement was taken. As in the first year of study, there was again high mortality for *O.c.* No *O.c.* survived for 2 years in captivity. Initially N = 13 for *O.a.p.* and N = 12 for *O.c.*

were also compared as a mean of a percent change (Figure 3B).

One group of stygobite crayfish was used for comparison of postorbital carapace length to total body length (Table 1). The postorbital carapace length accounts for about 40% of the total body length. The percent in the increase of growth, as measured by postorbital carapace length, is ca. 9% (Table 2). There did not appear to be a correlation with size of the animal and the percent increase in growth within a single molt.

DISCUSSION

The results of this study demonstrate that *O. a. packardi* can be maintained well in captivity under defined conditions for up to 2 years. This was the case for both small and large crayfish. However, *O. cristavarius* initially showed a good survival rate, which declined rapidly the longer they were housed, particularly after 7 months. Since small and large epigean crayfish died, the failure to survive was probably due to unsuitable conditions for this species within the laboratory rather than to senescence. This study also demonstrated that the epigean as well as the cave crayfish molted and grew in captivity. One approach to measure growth rates in crustaceans is to measure the time from one molt to the next in addition to size increases resulting from each molt. Only some of the crayfish we held molted a second time (Table 3). Based on the molt frequency, there is little or no difference in growth rate between the two species, with *O. a. packardi* having slightly more second molts. In some cases where a molt was documented the cave animals did not show more than a few millimeters increase in body length as assessed during the first year of study by total body length. With measures of postorbital carapace length, as used in the second year of study, an approximate increase in length with each molt is 9%, but there is considerable individual variation (Table 2).

Preliminary growth studies with the stygobitic crayfish *Orconectes inermis inermis* Cope 1872, in Pless Cave, Indiana, using a mark-and-recapture approach, indicated that juveniles showed larger increments of growth at ecdysis than mature animals (Hobbs III 1976). Using repetitive recaptures of hundreds of marked individuals of three species of stygobitic crayfish in Shelta Cave, Alabama, Cooper (1975) predicted that one of the species, *Orconectes australis australis* (Rhoades 1941), might live for 30 years or more. This was based on known minimum size at recruitment, maximum size, and growth increments observed following molts (Cooper and Cooper 1978, 1979; Culver 1982, p. 51). Detailed growth studies over a 1-year period of *Cambarus laevis* from epigean groups as well as groups that lived within the cave were conducted with recapture techniques. From such measures, growth curves were established (Weingartner 1977). In Weingartner's study a

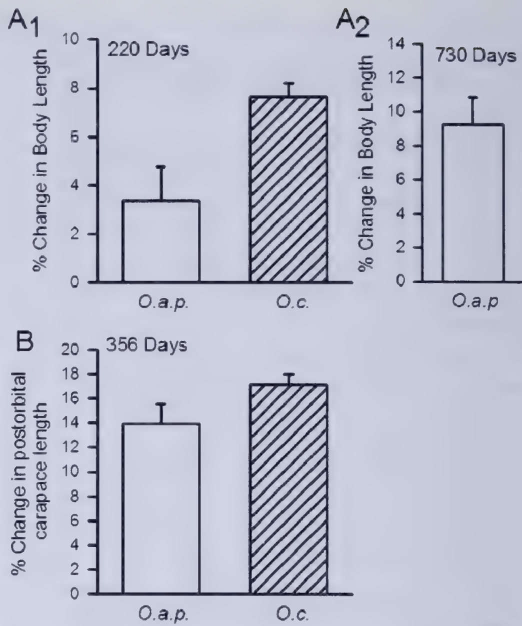

Figure 3. Comparisons in the growth between stygobitic (*Orconectes australis packardi*) and epigean (*Orconectes cristavarius*) crayfishes. (A_1) During the first year of study in which the total body lengths were used, a comparison in percent of growth between *Orconectes cristavarius* (*O.c.*) and *Orconectes australis packardi* (*O.a.p.*) is shown (N = 13 for *O.a.p.* and N = 10 for *O.c.*). Since so many *O.c.* died, the values obtained after 220 days were used for both species. The error bars represent the ± of the standard error of the mean. (A_2) The percent of growth for *O.a.p.* based on total body length, is shown after a 2-year period in captivity. Since no *O.c.* survived for 2-years a comparison could not be made. (B) In the second year of study the postorbital carapace length was used as an index of growth (N = 8 for *O.a.p.* and N = 3 for *O.c.*). The sample size is smaller for *O.c.* because of the lack in survival of this species.

Table 1. Comparison of total body length to postorbital carapace length for *Orconectes australis packardi*. Two additional animals not used in the growth studies were used for these morphometric measures.

Total body length (mm)	Postorbital carapace length (POL) (mm)	Ratio body/POL
43	16	0.37
44	18	0.41
47	17.5	0.37
50	20	0.40
51	20	0.39
55	22.5	0.41
58	19	0.33
60	23	0.38
60	23.5	0.39
71	29	0.41
71	28	0.39
Mean		0.39
SEM		0.024

Table 2. The percent change in POL within a molt for *Orconectes australis packardi*.

Premolt (mm)	Postmolt (mm)	% change
17.1	18.0	5.26
17.5	19.1	9.14
18.0	20.0	11.1
18.2	20.0	9.89
18.3	20.5	12.02
19.0	21.0	10.53
20.0	22.2	11.0
20.5	22.1	7.8
21.0	23.0	9.52
22.8	23.5	3.07
23.5	25.3	7.66
26.4	28.0	3.06
27.0	29.0	7.41
28.0	30.0	7.14
Mean		8.4
SEM		0.7

few animals were recaptured after 3 years and the growth curves were extended for that length of time; however with only a few animals the variability was not able to be assessed for differences in the age of the animals.

Poor survivorship for *O. cristavarius* in our studies is likely a species-dependent phenomenon, since another epigean crayfish, *Procambarus clarkii* (Girard 1852), from Raceland, Louisiana, survived well in the same laboratory conditions for 2 years or more. The reason for the difference in survival between these two epigean species may be a result of environmental adaptation, since *O. cristavarius* is predominantly found in fast-moving, highly oxygenated streams, and *P. clarkii* comes from swamps. It might be that the *P. clarkii* and *O. a. packardi* can survive well in water that is not highly oxygenated, such as that used in our laboratory, while *O. cristavarius* cannot. It is known that *P. clarkii* is very hardy and can tolerate all but the severest cases of hypoxia (McClain 1999). There might also be dietary factors that we did not investigate to account for the survival differences. All crayfishes held in captivity were fed the same diet at the same time. In addition, larger animals had a greater quantity of food provided to them.

Stygobitic crayfishes also show an amazing resistance to experimentally induced fluctuations in temperature, from freezing in blocks of ice to rapid exposure of high temperature (32.5°C) (Park et al. 1941). In addition, they are known to be starvation resistant, possibly due to a lower metabolic rate (Dickson and Franz 1980; Dickson and Giesy 1982). Burbanck et al. (1948) and Jegla (1964) both reported that cave crayfish have a lower metabolic rate as compared to epigean species. Comparable studies on *O. cristavarius* are lacking, so it remains unknown if this species can tolerate general stress as well as *P. clarkii* and *O. a. packardi*.

The differential in the initial growth rate between *O. cristavarius* and O. *a. packardi* is interesting. It is possible that the handling, transport, and exposure to a new environment are factors, although care was taken not to allow the animals to heat up in transport or be exposed to fluctuating temperature. However, a change in temperature from a surface stream or cave could have an impact on the initial molting frequency. Temperature is con-

Table 3. Comparison of growth rates by frequency of molts within the first and second years of captivity for *Orconectes australis packardi* and *Orconectes cristavarius* crayfishes.

	1st molt	2nd molt
1st year		
O.a.p.	5 of 12 animals	none
O.c.	10 of 12 animals	1 of 4
2nd year		
O.a.p.	14 of 8 animals	5 of 8 animals
O.c.	10 of 9 animals	3 of 3 animals

sidered to be one of the most significant factors in regulating crustacean growth (Conan 1985): not only an increase in water temperature but a reduction as well can induce molting. Other small crustaceans, such as copepods (Vidal 1980) and amphipods (Dagg 1976), reduce their molt frequency in cold temperatures, but when this occurs the animals grow larger after each molt than when they molt more frequently. In contrast, an increase in temperature can inhibit some crustaceans from molting (Haefner and van Engle 1975). The shrimp *Crangon crangon* Linnaeus is known to have varied longevity depending on environmental temperature (Labat 1977; Llyod and Yonge 1947; Oh 1999). For a review of factors regulating growth in crustaceans see Wenner (1985).

The increase in initial growth of *O. cristavarius* is not likely due to 'catch-up' growth, common in crustaceans when they are exposed to sufficient food after being deprived of food (Bostworth and Wolters 1995), since the habitat from which they were obtained was abundant in crayfish, fish, and snails as dietary resources. On the other hand, the lack of growth for *O. a. packardi* even after a molt might be expected since it has been reported that an *O. a. australis* measuring 35.2 mm in total carapace length did not alter its length after two molts (Cooper 1975). It would be of value to know if stygobitic crayfish do show 'catch-up' growth depending on resources, or if the growth attained for a molt is temperature regulated. If such growth does occur it would make it difficult to determine the correct age of adult animals without knowing their complete life history and environmental conditions. This is particularly relevant since Weingartner (1977) showed differential rates of growth within various regions in a single cave system. It was concluded that the environmental differences in water temperature and food resources likely accounted for the regional differences.

It is our hope that one will consider the possibility of long-term rearing of crayfishes in suitable laboratory conditions to gain insight into environmental factors regulating the developmental issues and to further refine laboratory conditions that promote survival.

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Vascular Flora of Five Reservoirs in the Berea College Forest, Madison and Jackson Counties, Kentucky

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ABSTRACT

A descriptive survey of the vascular plants from wetland and aquatic habitats of the five Berea College reservoirs, Madison and Jackson counties, in south-central Kentucky was conducted during the growing seasons of 1995, 1996, 1998, and 2003. Six plant habitats described are Vegetated Open Water, Emergent Marsh, Shrub Swamp, Seasonally Dewatered Mud and Sandflat, Sedge-grass Meadow, and Shallow Stream with Gravel Bar. Relative abundance values were determined for every taxon at each reservoir. The species list comprises 292 species, 175 genera, and 65 families. Thirty-eight taxa (13%) are exotics. Species are classified as Lycopodiophyta (1), Equisetophyta (2), Polypodiophyta (7), Pinophyta (1), and Magnoliophyta (281). In the National Wetland Classification, 205 species (70.0%) are obligate, facultative wetland, and facultative species.

INTRODUCTION

The Berea College Forest (BCF) consists of 3318 ha (8200 acres) in several tracts in parts of Madison, Jackson, and Rockcastle counties of south-central Kentucky. BCF is maintained for watershed/wilderness resources, timber management, wildlife management, recreation, and educational purposes by Berea College, Berea, Kentucky. In addition, the BCF has four reservoirs in Madison and Jackson counties that provide the water supply for the city of Berea and surrounding Madison County communities and one reservoir for flood-prevention purposes.

Prior to 1904, people from Berea obtained their water from wells or cisterns. In 1904, Berea College began collecting water from springs in the Berea College Forest and started the Berea College Waterworks. In 1919, Upper Silver Creek Reservoir was built to collect and store water for the city. A second reservoir, Lower Silver Creek Lake, was created in 1939 to provide more water. Cowbell Lake was built in 1954 because of increased water demand by Berea and south Madison County. As more water continued to be needed for Berea, its industrial areas, and south Madison County, Owsley Fork Reservoir was constructed in 1976. In 1986, Berea College was required to build an earthen dam to serve as a flood-retarding structure for the overflow or breach of the Cowbell Reservoir dam. This accumulated body of water is known as Red Lick Reservoir No. 2. These five reservoir lakes are managed and regulated by Berea College Utilities.

Ferren and Tonsor (1996) defined a reservoir as a pond or lake, natural or artificial, from which water may be withdrawn for irrigation and/or water supply. Our floristic study is a baseline inventory with emphasis on the wetland and aquatic flora from collections and observations at five man-made bodies of water, or lakes, which we have termed "reservoirs" for descriptive purposes. These bodies of water are classified under the Lacustrine System of Cowardin et al. (1979), which are characteristically bounded by uplands or by wetlands dominated by shrubs, persistent emergents, and emergent mosses and lichens.

Published botanical works for the Berea College Forest are Grossman and Pittillo (1962), Wade and Thompson (1990), and Thompson and Fleming (2004). Among these three studies, over 600 plant species have been collected from the forest. Recent wetland floristic studies in south-central and east-central Kentucky include Hoagland and Jones

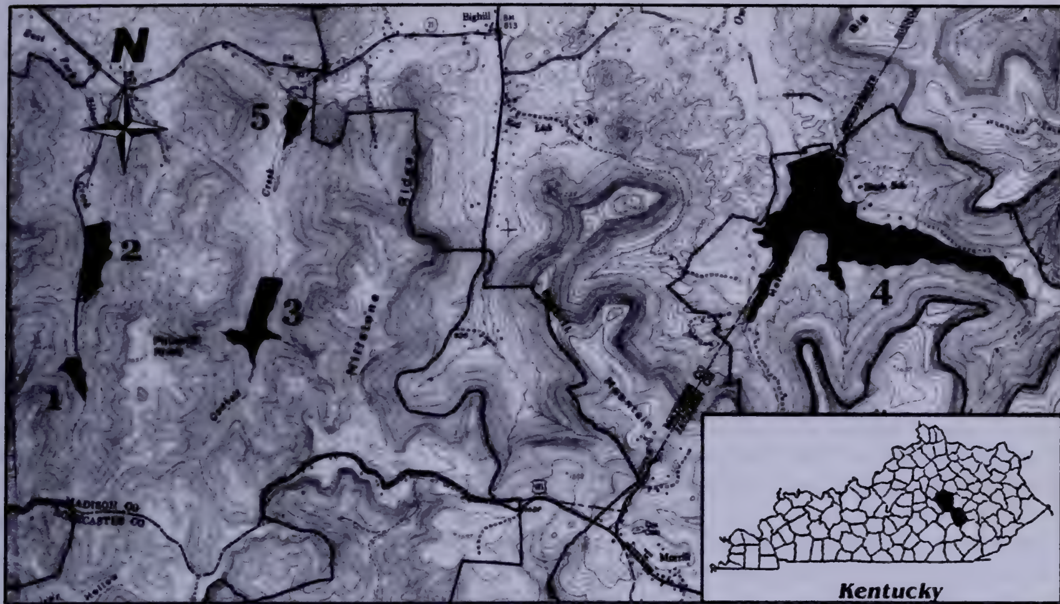

Figure 1. The five reservoirs within the Berea College Forest, Bighill Quadrangle, Kentucky, Photorevised 1979: (1) Upper Silver Creek Reservoir, (2) Lower Silver Creek Reservoir, (3) Cowbell Reservoir, (4) Owsley Fork Reservoir, and (5) Red Lick Reservoir No. 2. Kentucky inset with Madison (upper left) and Jackson (lower right) counties darkened.

(1992), Luken and Bezold (2000), and Thompson and FitzGerald (2003).

Baseline inventories of the vascular flora from reservoirs are important for preserving wetland areas, moderating the effects of floods, improving water quality, and enhancing aesthetic and heritage value (Mitsch and Gosselink 1993). Wetland studies have provided valuable information on soils, hydrological patterns, plant communities, hydrosere succession, and environmental controlling factors (Meagher and Tonsor 1992).

The objectives of our descriptive floristic survey were (1) to document the vascular flora with representative voucher specimens, (2) to ascertain plant origin (i.e., native or exotic), (3) to indicate National Wetland Classification Status for each taxon, (4) to determine relative abundance, and (5) to describe habitats for each species.

THE ENVIRONMENTAL COMPLEX

Reservoir Descriptions

Upper Silver Creek Reservoir (USC). Also known as Kale Lake or A-Lake, USC is the smallest reservoir, 2.1-ha surface area (5.3 acres), and was formed by damming the East Fork of Silver Creek. The concrete dam lies at 341 m elevation. USC is 10 m deep at its deepest point with visibility of 3.5 m. Upper Silver Creek reservoir is located in Madison County at the end of a gravel road 2 km south of KY 21 and 5 km west of the US 421 junction with KY 21 at Bighill (Figure 1).

Lower Silver Creek Reservoir (LSC). LSC (B-Lake or North Lake) has 7.8-ha surface area (19.3 acres). The north-trending concrete dam spillway lies at 298 m elevation. LSC is 10.5 m deep with visibility at 3 m. Lower Silver Creek Reservoir lies 0.3 km north of USC on the East Fork Silver Creek and 1.7 km south of KY 21 (Figure 1).

Cowbell Reservoir (CBR). CBR has 6.8-ha (16.8 acres) surface area and was formed by damming of the Cowbell Creek watershed. A north-trending earthen and concrete dam lies at 301 m elevation. CBR is 12 m deep with visibility of 3 m. Cowbell Reservoir is located in Madison County at the end of a gravel road 1.8 km south of KY 21 and 2.4 km west of junction US 421 and KY 21 at Bighill (Figure 1).

Owsley Fork Reservoir (OFR). This, the largest reservoir, has 61.1-ha (151 acres) surface area. OFR was formed by damming Owsley Fork Creek and Radford Hollow tributary. The north- and northwest-trending earthen and concrete dam lies on the Madison and Jackson county boundary at 250 m elevation. OFR has a maximum depth of 12 m with visibility of 3.5 m. Owsley Fork Reservoir is the only lake open to the general public, but with certain restrictions. OFR is located 4 km east of Bighill from the junction of US 421 with KY 21 which becomes Owsley Fork Road on the east side of OWF in Jackson County. A paved road, Radford Hollow Road, separates from Owsley Fork Road to the southwest of the dam on the Madison County side (Figure 1).

Red Lick Reservoir No. 2 (RLR). This reservoir of 3-ha (7.4 acres) was designed as a flood-retarding structure with an earthen dam formed from landfill from the adjacent mixed hardwood forest. The elevation of the dam top is 262 m. RLR is 5 m deep with limited visibility of 1 m. It is located 1.2 km north of Cowbell Reservoir on Cowbell Creek, and 0.7 km south of KY 21 (Figure 1).

Physiography and Geology

The Berea College Forest of Madison and Jackson counties lies within the Knobs Lower Scioto Dissected Plateau Region of the Western Allegheny Plateau and the Knobs Normal Upland Region of the Interior Plateau (Wood et al. 2002). The geological substrate within the area of the Berea College reservoirs is very complex. Drainage below the reservoirs has some Holocene Alluvium (210–230 m) of the Quaternary System, and the valleys have some New Albany Shale (230–275 m) of the Upper Devonian and Lower Mississippian System. Bedrock surrounding the reservoirs on lower slopes consists of shale, siltstone, and limestone of the Nancy Member (275–335 m), Cowbell Member (335–365 m), Nada Member (365–385 m), and Renfro Member (385–396 m). All these members belong to the Borden Formation of the Mississippian System (Weir et al. 1971). The middle and upper slopes and ridgetops of the Knobs consist of limestone and shale of Newman Limestone Member (396–450 m) and Pennington Formation (450–457 m) sandstone, siltstone and shale, both of the Upper Mississippian System. Several higher Knobs are capped with the Livingston Conglomerate Member (457–470 m) and/or the Corbin Sandstone Member (470–488 m) in the Lee Formation of the Lower Pennsylvanian System (Weir et al. 1971).

Soils

The majority of the forest soils surrounding the reservoirs belong to the the Weikert series or the Bledsoe-Gilpin-Shelocta-Grigsby series. The Weikert series are shallow, well-drained, moderately permeable soils with an acid pH from 4.5 to 5 derived mainly from acid siltstones (Newton et al. 1973). The Weikert soil series includes the majority of the soils surrounding Cowbell, Red Lick Flood Retarding Structure, Upper Silver Creek, and Lower Silver Creek reservoirs located entirely in Madison County. The soils of the Bledsoe-Gilpin-Shelocta-Grigsby series are typically deep, well-drained, and moderately permeable soils with a pH from 4.6 to 7. They are formed mainly in mixed alluvium from acid siltstones and shales (Hayes 1989). This soil association is found only at the Owsley Fork Reservoir study site in Jackson and Madison counties.

Climate

The climate of Kentucky is warm temperate, humid mesothermal, with little or no water deficiency, and is characterized by long warm summers and short mild winters (Trewartha and Horn 1980). The mean annual temperature of Berea is 13.7°C. July and August are usually the warmest months, with a mean temperature of 24.6°C, and January is the coldest month, with a mean temperature of 1.5°C. The mean annual precipitation is 119.3 cm with July having the highest precipitation at 12.2 cm and October the lowest with 5.8 cm. The mean frost-free growing season is 189 days with the mean date of the last spring freeze on 15 April and the first fall freeze on 22 October (Conner 1980).

Vegetation

Western Mesophytic Forest, a mosaic region of Mixed Mesophytic Forest and Oak-Hickory Forest, is the predominant vegetation in The Knobs Border Area as described by Braun (1950). Muller and McComb (1986)

classified the upland forests from eight sites within the Kentucky Knobs Region into mesophytic hardwoods, white oak, chestnut oak, and scarlet oak forest types. The upland forest bordering the Berea College reservoirs on the north- and northwest-trending aspects is primarily a mosaic of mesophytic hardwoods (*Liriodendron-Acer-Fagus-Quercus-Carya-Fraxinus*). The forest stands on the east-trending aspect and valley bottoms in the vicinity of the reservoirs are intermixed with mixed oak hardwoods-pine (*Quercus-Pinus*). All five reservoirs are surrounded by forests and open lands in various seral stages of secondary succession.

METHODS

The vascular flora was collected from the wetland habitats of the five reservoirs throughout the growing seasons of 1995, 1996, 1998, and 2003. Plants not in wetland habitats (i.e., contiguous secondary successional ecotones, upland forests, and reservoir dams) were not included in the species list. Manuals used for plant identification were Gleason and Cronquist (1991), Strausbaugh and Core (1978), and Beal and Thieret (1986). Plant classification and nomenclature are mostly from Gleason and Cronquist (1991). Representative voucher specimens were processed according to standard herbarium procedures and deposited in the Berea College Herbarium (BEREA). Previous plant collections from the BCF were also examined at BEREA.

Six wetland habitats modified from Thompson and FitzGerald (2003) were delineated from field reconnaissance of wetland habitats and species composition in these wetland areas from each reservoir. These wetland habitats are enclosed in brackets after the National Wetland Category in the Appendix. The six wetland habitats are (1) Vegetated Open Water (VOW)—an area of permanent water with a depth of up to 2 m with vegetation restricted to obligate free-floating species, floating-leaved submergents, and submergents; (2) Shrub Swamp (SS)—seasonally flooded area with saturated soils present when no standing water exists and dominated by riparian trees and shrubs with wetland herbaceous species; (3) Emergent Marsh (EM)—an area that is characterized by permanent or seasonal flooding or by water letdown, resulting in saturated soils during dry summers, and that supports obligate and facultative amphibious herbaceous plants; (4) Sedge-grass Meadow (SGM)—an area that has typically saturated soils covered with shallow water during times of increased rainfall and that supports obligate and facultative wetland herbs; (5) Seasonally Dewatered Mud-Sand Flat (SDF)—an area that is inundated for most of the year but with water receding early enough in summer, supporting annual species along exposed mudflats or sandy shoreline. The boundary between the seasonally dewatered flats and emergent marshes may vary from year to year based upon precipitation, evaporation, and water drawdown; and (6) Shallow Stream with Gravel Bar (SSG)—headwater inlet streams with vegetated streambanks, terraces, and gravel bar areas from periodical flooding; this habitat supports a rich assemblage of wetland and forest perennial herbs and woody plants. The forests and open lands in various seral stages of secondary succession surrounding the reservoirs were not inventoried for plants or included in the species list.

The Appendix includes the species name, plant origin (native or exotic), National Wetland Classification status, a representative wetland habitat for each taxon, and the relative abundance value of each species found at each reservoir.

An asterisk (*) preceding a species name denotes an exotic or non-indigenous plant species. After the species name is the National Wetland Category (Reed 1988). These National Wetland Categories are OBL = Obligate Wetland, FACW = Facultative Wetland, FAC = Facultative, FACU = Facultative Upland, and UPL = Upland, and may contain minus (−) or plus (+) designations for the drier or wetter limits of the facultative categories. A Not Categorized (NC) has been created for those species not listed or classified in the 1997 National Wetland Indicator Plant List, Northeastern Region 1 by the USFWS (1997) (Appendix).

In the Appendix, abbreviations horizontally from the earliest to most recently established are reservoirs are: USC = Upper Silver Creek, LSC = Lower Silver Creek, CBR = Cowbell, OFR = Owsley Fork, and RLR = Red Lick Reservoir No. 2. Relative abundance values are listed under the columns for each reser-

Table 1. Classification of vascular plants at Five Reservoirs in the Berea College Forest, Kentucky.

Division	Families	Genera	Species	Native	Exotic	Species composition (%)
Equisetophyta	1	1	2	2	0	0.69
Lycopodiophyta	1	1	1	1	0	0.34
Polypodiophyta	3	5	7	7	0	2.40
Pinophyta	1	1	1	1	0	0.34
Magnoliophyta	59	167	281	243	38	96.23
Magnoliopsida	44	125	190	167	23	65.07
Liliopsida	15	42	91	76	15	31.16
Totals	65	175	292	254	38	100.00

voir. Relative abundance values adapted from Thompson and Jones (2001) are R = Rare—1 to 5 individuals or colonies; I = Infrequent—6 to 30 individuals or colonies; O = Occasional—31 to 100 individuals or colonies; F = Frequent—hundreds of individuals or colonies; and A = Abundant—thousands of individuals or colonies (Appendix).

RESULTS

Taxonomic Summary

The documented vascular flora of the five Berea College Reservoirs comprises 292 species within 175 genera from 65 families. The annotated catalogue of plant species is composed of Lycopodiophyta (1), Equisetophyta (2), Polypodiophyta (7), Pinophyta (1), and Magnoliophyta (281). Thirty-eight species (13%) are exotics (Table 1). Two hundred five (70.0%) are OBL, FACW, and FAC wetland species (Appendix). The number of species in the wetland categories are OBL (61), FACW (73), FAC (70), FACU (61), UPL (11), and NC (16). Total species for each reservoir are USC (114), LSC (177), CBR (150), OFR (214), and RLR (135) (Appendix). The largest families in species are Asteraceae (48), Poaceae (35), Cyperaceae (24), Lamiaceae (13), Fabaceae (12), Polygonaceae (12), and Juncaceae (9). The largest genera are *Polygonum* (11), *Juncus* (9), *Panicum* (9), *Aster* (8), and *Carex* (8) (Appendix). *Polygonum densiflorum* is documented as a new Kentucky record.

Reservoir Flora and Habitats

The five reservoirs have developed various wetland habitats through hydrarch or hydrosere succession. Characteristic species and locally rare taxa are listed for each reservoir habitat. There is some zone intergradation in the species from the six habitats, some more conspicuous then others; e.g., a gradient or continuum exists between species among the EM, SS, and SDF habitats at Owsley Fork Reservoir.

The flora and habitats of the five reservoirs in the Berea College Forest—Upper Silver Creek, Lower Silver Creek, Cowbell, Owsley Fork, and Red Lick Reservoir No. 2—are described from the oldest to the earliest created body of water.

Upper Silver Creek Reservoir. The four habitats of USC are VOW, EM, SDF, and SGM. Important submerged vegetation in the VOW are *Najas guadalupensis, N. minor,* and *Potamogeton nodosus.* The EM lies in steep areas surrounding the reservoir and at a small area by the dam spillway. Generally, the EM and SDF habitats are heavily shaded by the encroaching upland vegetation. Characteristic species of the EM include *Alisma subcordatum, Cyperus strigosus, Juncus acuminatus, J. effusus* var. *solutus, Leersia oryzoides, Ludwigia alternifolia, Scirpus cyperinus,* and *Scutellaria lateriflora.*

The SDF occurs as small slough delta areas at the northwest shore and east shore, which are created from side ravine stream siltation and water drawdown. Typical taxa of this habitat include *Bidens* spp., *Boehmeria cylindrica, Impatiens capensis, Ludwigia alternifolia, Lycopus virginicus, Mimulus alatus,* and *Penthorum sedoides.* At the north end on the lower part of the dam, a small wet-meadow seepage exists. Some SGM species include *Apocynum cannabinum, Carex frankii, C. lurida, C. vulpinoidea, Eupatorium perfoliatum, E. serotinum, Linum striatum, Polygala sanguinea, Scirpus atrovirens,* and *S. pendulus. Scirpus polyphyllus* is restricted to USC.

The USC flora is represented by 27 OBL, 27 FACW, 26 FAC, 25 FACU, 4 UPL, and 5 NC species.

Lower Silver Creek Reservoir. The five wetland habitats of LSC are VOW, EM, SDF, SS, and SGM. The VOW habitat contains *Najas guadalupensis, N. minor,* and *Potamogeton nodosus.* The EM zone occurs at the northwestern end by the concrete spillway, extensively at the southern end, and in small areas along the west-and-east trending banks. Char-

acteristic emergents are *Eleocharis quadrangulata, Juncus acuminatus, Juncus effusus* var. *solutus, Leersia oryzoides, Scirpus cyperinus, S. validus,* and *Typha latifolia.* Other EM taxa include *Acorus calamus, Asclepias incarnata, Carex* spp., *Hypericum mutilum, Ludwigia alternifolia, L. palustris, Lycopus virginicus, Onoclea sensibilis,* and *Panicum rigidulum.*

The SDF habitat nearly encompasses the entire reservoir during times of water drawdown except at the concrete dam. Characteristic species are *Bidens* spp., *Boehmeria cylindrica, Fimbristylis autumnalis, Impatiens capensis, Lycopus virginicus, Microstegium vimineum, Mimulus alatus, M. ringens, Polygonum* spp., *Scirpus atrovirens,* and *Xanthium strumarium.* A small zone of SS behind the EM at the south end includes scattered *Platanus occidentalis, Salix exigua, S. nigra, S. sericea,* and *Sambucus canadensis.* The herbaceous layer of SS mainly comprises species present in the seasonally dewatered flats and emergent marsh. The SGM habitats in the southern terminus and the northern end comprise the most floristically rich habitat. Characteristic SGM species include *Agrimonia parviflora, Asclepias incarnata, Aster dumosus, Carex frankii, C. lurida, C. tribuloides, C. vulpinoidea, Eupatorium coelestinum, E. fistulosum, E. perfoliatum, Euthamia graminifolia, Helianthus angustifolius, Lobelia cardinalis, L. siphilitica, Panicum clandestinum, Polygala sanguinea, Rhexia virginica,* and *Spiranthes cernua.*

Wetland species found only at LSC are *Acorus calamus, Aster puniceus, Carex squarrosa, C. stipata, Cyperus brevifolioides, Habenaria flava, H. lacera, H. peramoena, Lilium canadense, Pluchea camphorata,* and *Solidago rugosa* (Appendix).

The LSC flora is represented by 36 OBL, 50 FACW, 40 FAC, 36 FACU, 5 UPL, and 10 NC species.

Cowbell Reservoir. Four wetland habitats at CBR are classified as VOW, EM, SDF, and SS. The perimeter is entirely forested with steep terrain on both the east and west-trending banks. *Najas guadalupensis, Potamogeton diversifolius, P. nodosus,* and *P. pusillus,* are found in the VOW zone. The EM at the south and southwest watershed area supports typical hydrosere successional plants. Characteristic emergent species are *Eleocharis quadrangulata, Juncus effusus* var. *solutus, Leersia oryzoides, Panicum rigidulum, Scirpus cyperinus, S. pendulus,* and *Typha latifolia.* The SDF zone is located around shoreline especially on the west and south sides. Characteristic species along the SDF shoreline are *Boehmeria cylindrica, Carex vulpinoidea, Impatiens capensis, I. pallida, Juncus tenuis, Lycopus virginicus, Polygonum cespitosum* var. *longisetum, Polygonum punctatum,* and *Rotala ramosior.*

Shrub swamp habitats are located on the east and west banks at CBR. Indicator woody trees are *Acer negundo, A. saccharinum, Betula nigra, Platanus occidentalis, Populus deltoides,* and *Salix nigra.* Characteristic shrubs are *Hydrangea arborescens, Lindera benzoin,* and *Sambucus canadensis. Toxicodendron radicans* is the most prevalent woody vine. Herbaceous plants in the SS differ little in species composition from those found in the surrounding EM and SDF habitats.

Wetland species, *Betula nigra, Gratiola neglecta, Populus deltoides, Potamogeton diversifolius, P. pusillus,* and *Thelypteris hexagonoptera,* are recorded only from CBR (Appendix).

The CBR flora consists of 34 OBL, 35 FACW, 34 FAC, 35 FACU, 4 UPL, and 8 NC species.

Owsley Fork Reservoir. All six wetland habitats—VOW, EM, SDF, SS, SGM, and SSG—are present at OFR. Characteristic species in the VOW are *Najas guadalupensis, N. minor, Potamogeton illinoensis,* and *P. nodosus.* Two large areas of EM are present at OWF; one located at the western shore of Madison County and the other at the southeastern shore of Jackson County. Characteristic emergents are *Alisma subcordatum, Eleocharis palustris, E. quadrangulata, Equisetum arvense, E. hyemale, Galium tinctorium, Juncus effusus* var. *solutus, Ludwigia alternifolia, Sagittaria australis, Scirpus purshianus, S. validus, Typha angustifolia,* and *T. latifolia* (Appendix).

The Seasonally Dewatered Mud-Sand Flats encircle the entire lake in the southwest and southeast coves of the reservoir where the shoreline is much more level, exposed, and drier during water letdown than the shaded north-trending aspect. Characteristic species of the SDF are *Bidens* spp., *Cyperus strigosus, Diodia virginiana, Eclipta prostrata, Eleo-*

charis ovata, Lindernia dubia, Ludwigia palustris, Lysimachia nummularia, Penthorum sedoides, Polygonum spp., *Rotala ramosior, Scirpus purshianus, Scutellaria lateriflora,* and *Xanthium strumarium.*

Shrub swamp habitats serve as an intergrading boundary between the SDF and EM zones in the largest coves of Owsley Fork Reservoir. Woody species of the Jackson county SS in the eastern corner of the lake are *Acer negundo, A. saccharinum, Cornus drummondii, Platanus occidentalis, Salix exigua, S. nigra,* and *S. sericea.* Herbaceous wetland species principally include those of the SGM and SDF zones. The SS in the northwest corner of the reservoir in Jackson County has nearly the same floristic composition in the Madison County side.

Owsley Fork Reservoir has a large SGM on the north side in Jackson County and also on the east side in Madison County. The SGM species include *Agalinis purpurea, Agrimonia parviflora, Apocynum cannabinum, Asclepias incarnata, Eupatorium coelestinum, E. fistulosum, E. perfoliatum, Impatiens capensis, Juncus* spp., *Mentha* ×*piperita,* and *Scirpus atrovirens.* The SSG zones were present at the watershed of the SS of both Madison and Jackson counties where intermittent streams are located. Characteristic woody taxa include *Campsis radicans, Clematis virginiana, Hydrangea arborescens, Lindera benzoin, Sambucus canadensis,* and *Toxicodendron radicans.* On the streambanks and gravel bars are found *Bidens* spp., *Boehmeria cylindrica, Equisetum arvense, Impatiens capensis, I. pallida, Lobelia siphilitica,* and *Microstegium vimineum.*

Wetland species restricted to OFR are *Cicuta maculata, Cornus drummondii, Eleocharis tenuis, Epilobium coloratum, Equisetum hyemale, Galium tinctorium, Juncus brachycarpus, J. diffusissimus, J. torreyi, Leucospora multifida, Lycopus americanus, Lysimachia nummularia, Potamogeton illinoensis, Sagittaria calycina,* and *Typha angustifolia.*

The OFR flora is made up of 47 OBL, 55 FACW, 51 FAC, 42 FACU, 10 UPL, and 9 NC species (Appendix).

Red Lick Reservoir No. 2. RLR has all six wetland habitats—VOW, EM, SDF, SS, SGM, and SSG. Characteristic plants of the VOW habitat are *Brasenia schreberi, Lemna minor, Najas guadalupensis,* and *Potomogeton nodosus.* The EM at this reservoir lies along the east-facing bank and the southern end of the lake. Smaller areas of this habitat can also be found in the northwest corner near the dam and along the steep, west-trending bank of undisturbed mesophytic hardwood forest. The EM zone is dominated by *Eleocharis quadrangulata, Juncus effusus* var. *solutus, Leersia oryzoides, Panicum rigidulum, Polygonum densiflorum, Scirpus cyperinus, Sparganium americanum,* and *Typha latifolia.*

The SDF habitats are located in the south end between the SS and the EM and in a few other small sites depending on the seasonally evaporated water levels. Characteristic species include *Cyperus strigosus, Eleocharis ovata, Fimbristylis autumnalis, Impatiens capensis, Lindernia dubia* var. *anagallidea, Ludwigia alternifolia, Penthorum sedoides, Rotala ramosior,* and *Xanthium strumarium.*

A small SS zone at the south end near the SSG entrance and on the disturbed west shore has *Alnus glutinosa, Cornus amomum, Liquidambar styraciflua, Platanus occidentalis,* and *Salix nigra.* Herbaceous plants are primarily those of the SDF and SGM. The SGM along the west side includes *Boehmeria cylindrica, Carex lurida, C. tribuloides, Equisetum arvense, Helianthus angustifolius, Lobelia siphilitica, Lythrum salicaria, Mimulus alatus, Pilea pumila,* and several other species found mainly on the SDF. The SSG habitat, located at the southern terminus of the reservoir, is floristically different from any of the other habitats. The SSG streambanks include *Carpinus caroliniana, Hydrangea arborescens, Liquidambar styraciflua,* and *Sambucus canadensis*; gravel bars support *Boehmeria cylindrica, Carex torta, Equisetum arvense, Glyceria striata,* and *Impatiens capensis.*

Alnus glutinosa, Brasenia schreberi, Carex torta, Cornus amomum, Lemna minor, Ludwigia decurrens, Liquidambar styraciflua, Lythrum salicaria, Panicum verrucosum, Polygonum densiflorum, and *Sparganium americanum* are wetland species found only at RLR.

The RLR flora is composed of 35 OBL, 34 FACW, 32 FAC, 26 FACU, 3 UPL, and 5 NC species (Appendix).

DISCUSSION

The five Berea College reservoirs exhibit similar stages of hydrosere succession composed of characteristic wetland species in specific wetland habitats. Each reservoir varies in development of plant habitats and species composition based in part on factors of reservoir age, size and depth, existing vegetation contiguous to the lakes, hydrarch succession, and copper sulfate algicide treatments. The four reservoirs used for utilities water are currently mesoligotrophic. Although Red Lick Reservoir No. 2 is the most recently formed reservoir, it has advanced to a more eutrophic stage than the older reservoirs. Some contributing factors include sedimentation from adjacent reclaimed land, shallow depth, and a lack of direct copper sulfate treatments.

The species richness of wetland and aquatic vascular plants appears to be high with 70% belonging to the OBL, FACW, and FAC categories. This is a significant percentage considering that the upland forested and open terrain is predominately composed of FACU and UPL species. Each reservoir has transitional zones of upland forest and grassy areas bordering the wetland habitats. Many facultative species have taken advantage of the moisture regimens of these reservoirs. Red Lick Reservoir No. 2 has a species richness comparable to the other four reservoirs in total plant species including its number of wetland and aquatic plants.

Hydrarch succession, a type of progressive secondary succession, has been induced by a number of factors at the reservoirs. The first of these factors may have been the presence of viable seed banks prior to reservoir construction. Once these diaspores were given the appropriate environmental conditions for germination and subsequent growth, they became established as various habitats developed. Water and wind are important dispersal mechanisms for propagules. The flora and fauna have shaped the floristic composition in a number of ways. Animals have aided in the distibution of viable seeds to account for a greater species richness. Waterfowl, for example, could be responsible for certain wetland species that clearly were not present prior to lake formation, e.g., *Acorus calamus, Brasenia schreberi, Lemna minor, Sagittaria calycina,* and *Sparganium americanum.* The flora allows for the formation of better-suited habitats for wetland species, through decaying of plant material, resulting in the addition of organic material to the soil, and through increased evapotranspiration, allowing for areas with drier soils (Mitsch and Gosselink 1993). These factors have increased the ability of these areas to support various degrees of high species richness.

Several wetland and aquatic species are locally rare in the wetland habitats of the five reservoirs. These taxa often are restricted to a single reservoir site, i.e., *Habenaria flava, H. lacera,* and *H. peramoena* at Lower Silver Creek Reservoir. We encountered no threatened, endangered, or special concern taxa for Kentucky (KSNPC 2000). We did document the presence of *Polygonum densiflorum,* a new Kentucky distribution record. Overall, 292 species have become established in the reservoirs within 10 to 77 years.

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Appendix. Vascular plants of the five Berea College Reservoirs, Madison and Jackson counties, Kentucky.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
Equisetophyta					
Equisetaceae					
Equisetum arvense L. FAC. [SSG]	—	O	O	F	O
E. hyemale L. FACW. [SS]	—	—	—	F	—
Lycopodiophyta					
Selaginellaceae					
Selaginella apoda (L.) Fern. FACW. [SSG]	—	I	—	I	—
Polypodiophyta					
Aspleniaceae					
Athyrium filix-femina (L.) Roth. FAC. [SSG]	R	—	—	—	R
A. pycnocarpon (Spreng.) Tidest. FAC. [SSG]	R	—	—	—	—
A. thelypterioides (Michx.) Desv. FAC. [SSG]	I	—	—	—	—
Polystichum acrostichoides (Michx.) Schott. FACU−. [SSG]	I	I	O	R	—
Thelypteris hexagonoptera (Michx.) Weatherby. FAC. [SSG]	—	—	I	—	—
Onocleaceae					
Onoclea sensibilis L. FACW. [SGM]	—	O	I	—	—
Ophioglossaceae					
Botrychium dissectum Spreng. FAC. [SDF]	—	R	R	—	—
Pinophyta					
Taxodiaceae					
Taxodium distichum (L.) Rich. OBL. [SDF]	—	—	—	I	—
Magnoliophyta					
Aceraceae					
Acer negundo L. FAC+. [SDF]	—	—	I	I	—
A. rubrum L. FAC. [SSG]	I	I	I	O	—
A. saccharinum L. FACW [SS]	—	—	I	I	—
Acoraceae					
Acorus calamus L. OBL. [EM]	—	I	—	—	—
Alismataceae					
Alisma subcordatum Raf. OBL. [EM]	I	I	O	F	I
Sagittaria australis (J.G. Smith) Small. OBL. [EM]	R	O	I	R	O
S. calycina Engelm. OBL. [EM]	—	—	—	O	—
Anacardiaceae					
Rhus copallina L. FACU−. [SSG]	—	I	—	—	I
R. glabra L. NC. [SGM]	—	I	—	—	—
Toxicodendron radicans (L.) Kuntze. FAC. [SDF]	O	F	F	O	O
Apiaceae					
Cicuta maculata L. OBL. [SGM]	—	—	—	R	—
Cryptotaenia canadensis (L.) DC. FAC. [SGM]	I	—	I	I	—
**Daucus carota* L. NC. [SGM]	I	O	I	O	I
Osmorhiza claytonii (Michx.) Clarke. FACU−. [SGM]	R	—	I	R	—
Sanicula canadensis L. UPL. [SGM]	R	O	I	I	—
Apocynaceae					
Apocynum cannabinum L. FACU. [SGM]	O	O	O	F	I
Asclepiadaceae					
Ampelamus albidus (Nutt.) Britt. FAC. [SDF]	—	—	—	I	—
Asclepias incarnata L. OBL. [SDF]	I	O	I	F	I
A. syriaca L. FACU−. [SGM]	I	I	I	I	R
A. tuberosa L. NC. [SGM]	—	R	—	R	—
A. viridis Walt. NC. [SGM]	R	R	—	—	—
Asteraceae					
**Achillea millefolium* L. FACU. [SGM]	I	O	I	—	—
Ambrosia artemisiifolia L. FACU. [SGM]	O	O	I	O	O
A. trifida L. FAC. [SGM]	—	O	—	O	O
Aster cordifolius L. NC. [SSG]	O	O	I	I	I
A. dumosus L. FAC. [SGM]	—	O	I	I	—
A. divaricatus L. NC. [SSG]	—	I	I	I	—
A. lateriflorus (L.) Britt. FACW−. [SDF]	I	I	I	I	—
A. ontarionis Wieg. FAC. [SDF]	—	—	—	I	I

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
A. pilosus Willd. UPL. [SGM]	—	I	—	I	I
A. prenanthoides Muhl. FAC. [SDF]	—	—	—	I	I
A. puniceus L. OBL. [SDF]	—	R	—	—	—
Bidens cernua L. OBL. [SDF]	—	O	I	I	—
B. frondosa L. FACW. [SGM]	I	I	O	O	O
B. polylepis S. F. Blake. FACW. [SGM]	I	O	I	O	O
**Chrysanthemum leucanthemum* L. UPL. [SGM]	—	—	—	I	I
Conyza canadensis (L.) Cronq. UPL. [SGM]	—	—	I	—	—
**Eclipta prostrata* (L.) L. FAC. [SDF]	R	—	—	I	—
Elephantopus carolinianus Willd. FACU. [SGM]	—	—	O	—	—
Erechtites hieracifolia (L.) Raf. FACU. [SGM]	—	—	R	R	R
Eupatorium coelestinum L. FAC. [SGM]	—	O	I	O	I
E. fistulosum Barratt. FACW. [SDF]	I	F	—	O	O
E. purpureum L. FAC. [SGM]	—	R	—	—	—
E. perfoliatum L. FACW+. [SGM]	I	O	I	F	O
E. rotundifolium L. FAC−. [SGM]	—	I	—	—	—
E. serotinum Michx. FAC−. [SGM]	I	O	O	O	O
Euthamia graminifolia (L.) Nutt. FAC. [SGM]	—	O	O	O	—
Helenium flexuosum Raf. FAC−. [SDF]	—	I	—	I	—
Helianthus angustifolius L. FACW. [SDF]	—	O	—	—	O
Helianthus microcephalus T. & G. NC. [SGM]	—	—	R	—	—
Iva annua L. FAC. [SGM]	—	—	—	—	I
Lactuca canadensis L. FACU−. [SGM]	I	O	O	I	R
Pluchea camphorata (L.) DC. FACW. [SDF]	—	—	R	—	—
Polymnia uvedalia L. FAC. [SGM]	—	—	R	I	—
Prenanthes altissima L. FACU−. [SGM]	—	—	I	—	—
Pyrrhopappus carolinianus (Walt.) DC. NC. [SGM]	—	I	R	R	—
Rudbeckia fulgida Ait. FAC. [SDF]	—	—	R	—	—
R. hirta L. FACU. [SGM]	—	—	I	—	—
R. triloba L. FACU. [SGM]	—	R	—	R	—
Silphium trifoliatum L. FAC. [SGM]	—	I	—	I	—
Solidago caesia L. FACU. [SDF]	O	I	I	O	I
S. canadensis L. FACU. [SGM]	—	F	—	O	I
S. flexicaulis L. FACU. [SDF]	O	O	O	I	—
S. gigantea Ait. FACW. [SDF]	—	—	R	O	—
S. rugosa P. Mill. FAC. [SGM]	—	O	—	—	—
**Sonchus asper* (L.) Hill. FAC. [SGM]	—	—	—	—	R
Verbesina alternifolia (L.) Britt. FAC. [SGM]	—	O	—	O	—
Vernonia gigantea (Walt.) Trel. FAC. [SGM]	—	I	—	I	—
Xanthium strumarium L. FAC. [SDF]	I	O	O	F	I
Balsaminaceae					
Impatiens capensis Meerb. FACW. [SGM]	F	F	F	A	F
I. pallida Nutt. FACW. [SSG]	—	—	I	I	I
Betulaceae					
**Alnus glutinosa* (L.) Gaertn. FACW−. [SS]	—	—	—	—	I
Betula nigra L. FACW. [SS]	—	—	R	—	—
Carpinus caroliniana Walt. FAC. [SS]	—	—	—	I	I
Corylus americana Walt. FACU−. [SWG]	—	—	—	I	I
Bignoniaceae					
Campsis radicans (L.) Seem. FAC. [SSG]	—	R	I	O	I
Brassicaceae					
**Cardamine hirsuta* L. FACU. [SGM]	R	—	R	—	—
Lepidium virginicum L. FACU−. [SGM]	—	—	—	I	—
Rorippa palustris (L.) Bess. OBL. [EM]	—	—	I	O	—
Cabombaceae					
Brasenia schreberi J. F. Gmel. OBL. [VOW]	—	—	—	—	F
Campanulaceae					
Campanula americana L. FACU. [SGM]	—	—	—	I	—
Lobelia cardinalis L. FACW+. [SGM]	—	R	—	I	—
L. inflata L. FACU. [SDF]	I	I	I	O	I

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
L. puberula Michx. FACW−. [SDF]	—	I	—	I	—
L. siphilitica L. FACW+. [SGM]	I	O	I	O	I
L. spicata Lam. FAC−. [SGM]	—	R	—	—	—
Triodanis perfoliata (L.) Nieuwl. FAC. [SGM]	I	—	—	—	—
Caprifoliaceae					
**Lonicera japonica* Thunb. FAC−. [SGM]	O	O	F	F	O
Sambucus canadensis L. FACW. [SWG]	I	O	I	O	I
Viburnum rufidulum Raf. UPL. [SDF]	—	—	R	—	—
Clusiaceae					
Hypericum mutilum L. FACW. [SDF]	I	O	O	O	O
H. punctatum Lam. FAC−. [SDF]	I	I	—	I	I
Commelinaceae					
**Commelina communis* L. FAC−. [SGM]	—	—	I	I	I
Convolvulaceae					
Calystegia sepium (L.) R. Br. FAC−. [SGM]	—	—	I	I	—
**Ipomoea lacunosa* L. FACW. [SDF]	I	O	—	O	I
I. pandurata (L.) G.Mey. FACU. [SGM]	—	I	I	—	—
Cornaceae					
Cornus amomum P. Mill. FACW. [SS]	—	—	—	—	R
C. drummondii C. A. Mey. FAC. [SS]	—	—	—	R	—
Cuscutaceae					
Cuscuta gronovii Willd. NC. [EM]	—	—	—	I	I
C. indecora Choisy. NC. [SDF]	—	I	I	—	—
C. pentagona Engelm. NC. [SGM]	—	O	—	O	R
Cyperaceae					
Carex frankii Kunth. OBL. [EM]	I	I	I	O	I
C. hirsutella Mack. FACU. [SDF]	—	I	—	—	—
C. lurida Wahl. OBL. [EM]	R	F	I	F	O
C. squarrosa L. FACW. [EM]	—	I	—	—	—
C. stipata Willd. OBL. [EM]	—	R	—	—	—
C. torta F. Booth. FACW. [SSG]	—	—	—	—	O
C. tribuloides Wahl. FACW+. [EM]	R	I	—	O	I
C. vulpinoidea Michx. OBL. [EM]	I	F	O	O	O
Cyperus brevifolioides Thieret & Delahoussaye FACW. [SGM]	—	I	—	—	—
C. flavescens L. OBL. [SDF]	—	I	—	R	—
C. strigosus L. FACW. [EM]	I	O	O	O	O
Eleocharis ovata (Roth) R. & S. OBL. [SDF]	I	F	F	F	O
E. palustris (L.) R. & S. OBL. [EM]	—	—	I	F	—
E. quadrangulata (Michx.) R. & S. OBL. [EM]	—	F	F	O	F
E. tenuis (Willd.) Schult. FACW+. [SDF]	—	—	—	O	—
Fimbristylis autumnalis (L.) R. & S. FACW+. [SDF]	—	O	—	O	O
Rhynchospora capitellata (Michx.) Vahl. OBL. [EM]	—	—	—	—	I
Scirpus atrovirens Muhl. OBL. [SDF]	I	I	I	F	R
S. cyperinus (L.) Kunth. FACW+. [SDF]	O	F	F	F	O
S. pendulus Muhl. OBL. [EM]	I	—	I	O	R
S. polyphyllus Vahl. OBL. [EM]	R	—	—	—	—
S. purshianus Fern. OBL. [EM]	—	—	I	F	—
S. validus Vahl. OBL. [EM]	—	F	—	F	F
Scleria triglomerata Michx. FAC. [SGM]	—	—	—	—	I
Dioscoreaceae					
**Dioscorea batatas* Dcne. NC. [SSG]	I	O	—	O	I
Euphorbiaceae					
Acalypha rhomboidea Raf. FACU−. [SDF]	—	—	—	O	I
A. virginica L. FACU−. [SDF]	—	—	—	R	—
Euphorbia maculata L. FACU−. [SDF]	—	R	R	O	—
E. nutans Lagasca. FACU−. [SDF]	—	I	I	O	I
Phyllanthus caroliniensis Walt. FAC+. [SDF]	—	—	R	R	—
Fabaceae					
Amphicarpaea bracteata (L.) Fern. FAC. [SGM]	O	F	O	F	O
Apios americana Medik. FACW. [EM]	—	—	—	I	O

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
Chamaecrista fasciculata (Michx.) Greene. FACU. [SGM]	—	O	—	F	I
Desmanthus illinoensis (Michx.) MacMill. FAC. [SGM]	I	O	—	—	—
Desmodium paniculatum (L.) DC. UPL. [SGM]	O	F	—	O	—
**Lespedeza cuneata* (Dum Cours.) Don. FACU−. [SDF]	I	O	I	O	O
L. intermedia (S. Wats.) Britt. NC. [SGM]	—	—	O	—	—
**L. stipulacea* Maxim. FACU. [SDF]	—	F	O	—	—
**L. striata* (Thunb.) H. & A. FACU. [SDF]	—	—	I	I	—
**Melilotus alba* Desr. FACU−. [SGM]	—	—	I	—	R
Strophostyles umbellata (Muhl.) Britt. FACU−. [SGM]	—	I	—	I	—
**Trifolium pratense* L. FACU−. [SGM]	—	F	—	—	—
Gentianaceae					
Sabatia angularis (L.) Pursh. FAC+. [SDF]	I	O	R	I	—
Hamamelidaceae					
Liquidambar styraciflua L. FAC. [SS]	—	—	—	—	I
Hydrangeaceae					
Hydrangea arborescens L. FACU. [SSGF]	I	O	O	O	I
Juncaceae					
Juncus acuminatus Michx. OBL. [EM]	I	O	O	O	O
J. biflorus Ell. FACW. [SGM]	—	—	R	O	—
J. brachycarpus Engelm. FACW. [SGM]	—	—	—	I	—
J. diffusissimus Buckl. FACW. [SGM]	—	—	—	O	—
J. dudleyi Wieg. FAC−. [SGM]	—	—	—	—	R
J. effusus L. var. *solutus* Fern. & Wieg. OBL. [EM]	O	F	O	F	F
J. marginatus Rostk. FACW. [SDF]	—	I	I	O	I
J. tenuis Willd. FAC−. [SGM]	I	O	I	F	O
J. torreyi Cov. FACW. [SGM]	—	—	—	I	—
Lamiaceae					
Collinsonia canadensis L. FAC+. [SGM]	I	I	R	I	—
Lycopus americanus Muhl. OBL. [EM]	—	—	—	O	—
L. virginicus L. OBL. [SDF]	O	O	I	F	I
**Mentha* ×*piperita* L. FACW+. [SGM]	—	I	—	O	—
Physostegia virginiana (L.) Benth. FAC+. [SSG]	—	—	—	R	—
**Prunella vulgaris* L. FACU. [SGM]	I	O	R	O	I
Pycnanthemum tenuifolium Schrad. FACW. [SDF]	O	F	—	O	R
Scutellaria elliptica Muhl. NC. [SDF]	I	—	—	—	—
Scutellaria lateriflora L. FACW+. [SDF]	I	O	O	F	—
Stachys nuttallii Schuttw. FAC. [SGM]	—	—	—	I	—
S. tenuifolia Willd. FACW+. [SDF]	R	O	I	R	—
Teucrium canadense L. FACW−. [SDF]	—	I	—	—	—
Trichostema dichotomum L. NC. [SGM]	—	—	—	R	—
Lauraceae					
Lindera benzoin (L.) Blume. FACW−. [WM]	—	—	I	I	—
Lemnaceae					
**Lemna minor* L. OBL. [VOW]	—	—	—	—	F
Liliaceae					
**Allium vineale* L. FACU−. [SGM]	I	I	—	—	—
Lilium canadense L. FAC+. [SGM]	—	R	—	—	—
Linaceae					
Linum medium (Planch.) Britt. FACU. [SDF]	I	I	—	—	—
L. striatum Walt. FACW. [SDF]	O	F	F	O	—
Lythraceae					
Ammannia coccinea Rottb. OBL. [SDF]	—	—	R	—	R
**Lythrum salicaria* L. FACW+. [SGM]	—	—	—	—	R
Rotala ramosior (L.) Koehne. OBL. [SDF]	I	—	O	F	I
Magnoliaceae					
Liriodendron tulipifera L. FACU. [SS]	I	O	I	O	O
Melastomataceae					
Rhexia virginica L. OBL. [SDF]	—	O	—	O	—
Molluginaceae					
**Mollugo verticillata* L. FAC. [SDF]	—	—	—	O	—

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
Najadaceae					
**Najas minor* All. OBL. [VOW]	O	O	F	F	—
N. guadelupensis (Spreng.) Magnus. OBL. [VOW]	F	F	A	A	F
Fraxinus pennsylvanica Marsh. FACW. [SS]	—	I	I	I	—
Onagraceae					
Circaea lutetiana L. FACU. [SGM]	—	—	—	R	—
Epilobium coloratum Biehler. FACW+. [SDF]	—	—	—	O	—
Ludwigia alternifolia L. FACW+. [SDF]	O	F	I	O	I
L. decurrens Walt. OBL. [EM]	—	—	—	—	R
L. palustris (L.) Ell. OBL. [SDF]	O	F	F	F	F
Orchidaceae					
Habenaria flava (L.) R. Br. FACW. [SGM]	—	R	—	—	—
H. lacera (Michx.) Loud. FACW. [SGM]	—	R	—	—	—
H. peramoena A. Gray. FACW. [SGM]	—	R	—	—	—
Spiranthes cernua (L.) Rich. FACW. [SDF]	—	O	R	R	—
S. lacera (Raf.) Raf. FACU−. [SGM]	I	—	—	—	—
Plantaginaceae					
Plantago rugelii Dcne. FACU. [SGM]	O	—	—	O	—
Platanaceae					
Platanus occidentalis L. FACW−. [SS]	I	O	I	O	I
Poaceae					
**Agrostis gigantea* Roth. FACW. [SDF]	I	O	O	O	—
A. perennans (Walt.) Tuckerm. FACU. [SGM]	—	—	I	—	I
Andropogon virginicus L. FACU. [SGM]	—	O	I	O	R
Brachyelytrum erectum (Schreb.) Beauv. NC. [SGM]	—	—	O	—	—
Cinna arundinacea L. FACW+. [SDF]	—	R	O	—	—
**Digitaria ischaemum* (Schreb.) Muhl. UPL. [SDF]	—	I	—	O	—
**D. sanguinalis* (L.) Scop. FACU−. [SDF]	—	—	—	O	—
**Echinochloa crus-galli* (L.) Beauv. FACU. [SDF]	—	—	—	R	—
E. muricata (Beauv.) Fern. FACW+. [SDF]	I	I	I	I	O
**Eleusine indica* (L.) Gaertn. FACU−. [SDF]	—	—	—	O	—
Elymus hystrix L. UPL. [SSG]	—	—	I	I	—
E. virginicus L. FACW−. [SDF]	—	I	—	I	—
**Eragrostis pectinacea* (Michx.) Nees. FAC. [SDF]	—	—	—	O	—
**E. spectabilis* (Pursh) Steudel. UPL. [SGM]	—	—	—	I	—
**Festuca elatior* L. FACU−. [SGM]	I	I	R	I	I
Glyceria striata (Lam.) Hitchc. OBL. [EM]	I	O	O	O	O
**Holcus lanatus* L. FACU. [SGM]	—	O	—	—	—
Leersia oryzoides (L.) Swartz. OBL. [EM]	O	F	F	F	O
L. virginica Willd. FACW. [SDF]	O	F	O	—	O
**Microstegium vimineum* (Trin.) Camus. FAC. [SSG]	F	A	F	A	F
Muhlenbergia frondosa (Poir.) Fern. FAC. [SSG]	—	—	—	O	—
Panicum anceps Michx. FAC. [SGM]	I	F	—	F	O
P. clandestinum L. FAC+. [SDF]	I	F	F	F	O
P. dichotomiflorum Michx. FACW−. [SDF]	—	O	I	I	—
P. dichotomum L. FAC. [SDF]	F	F	F	F	F
P. flexile (Gattinger) Scribn. FACU. [SDF]	—	—	—	I	I
P. lanuginosum Ell. FAC. [SDF]	—	F	—	F	O
P. polyanthes Schult. FAC. [SDF]	O	F	O	F	I
P. rigidulum Nees. FACW+. [EM]	—	I	O	F	I
P. verrucosum Muhl. FACW. [SGM]	—	—	—	—	I
Paspalum laeve Michx. FAC+. [SGM]	—	O	O	F	O
**Setaria faberi* Herrm. UPL. [SGM]	R	O	—	I	I
S. geniculata (Lam.) P. Beauv. FAC. [SGM]	—	I	R	O	—
**Sorghum halepense* (L.) Pers. FACU. [SDF]	—	—	—	I	—
Tridens flavus (L.) A. Hitchc. FACU. [SDF]	—	O	—	O	—
Polygalaceae					
Polygala ambigua Nutt. UPL. [SGM]	R	—	—	I	—
P. sanguinea L. FACU. [SGM]	I	O	—	—	—

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
Polygonaceae					
Polygonum amphibium L. OBL. [EM]	—	F	—	F	O
**P. cespitosum* Blume. var. *longisetum* (De Bruyn) Stewart FACU−. [SDF]	I	O	O	F	I
P. densiflorum Meissn. OBL. [EM]	—	—	—	—	I
P. hydropiperoides Michx. OBL. [EM]	—	—	—	O	—
P. lapathifolium L. FACW+. [SDF]	—	R	—	I	—
P. pensylvanicum L. FACW. [SDF]	I	—	O	O	—
**P. persicaria* L. FACW. [EM]	—	—	O	I	I
P. punctatum Ell. OBL. [SDF]	I	O	O	O	I
P. sagittatum L. OBL. [SDF]	O	O	—	O	—
P. scandens L. FAC. [SDF]	—	—	—	O	—
P. virginianum L. FAC. [SDF]	—	—	I	I	R
**Rumex crispus* L. FACW. [SDF]	—	I	—	O	I
Potamogetonaceae					
Potamogeton. diversifolius Raf. OBL. [VOW]	—	—	R	—	—
P. illinoensis Morong. OBL. [VOW]	—	—	—	F	—
P. nodosus Poir. OBL. [VOW]	F	A	F	A	A
P. pusillus L. OBL [VOW]	—	—	I	—	—
Primulaceae					
**Lysimachia nummularia* L. FACW+. [SGM]	—	—	—	F	—
Samolus floribundus HBK. OBL. [SDF]	I	I	O	O	R
Ranunculaceae					
Clematis virginiana L. FAC. [SDF]	R	O	I	F	O
Thalictrum pubescens Pursh. FACW+. [SGM]	—	I	—	—	—
Rosaceae					
Agrimonia parviflora Ait. FACW. [SGM]	I	F	—	A	F
A. rostellata Wallr. FACU. [SGM]	—	I	—	I	—
Geum canadense Jacq. FACU+. [SGM]	I	I	—	O	R
Potentilla norvegica L. FACU. [SGM]	—	—	O	—	—
Rubus pensilvanicus Poir. FACU. [SGM]	—	O	O	F	I
Rubiaceae					
Diodia virginiana L. FACW. [SDF]	—	O	—	F	—
Galium tinctorium (L.) Scop. OBL. [EM]	—	—	—	F	—
G. triflorum Michx. FACU. [SGM]	I	O	I	O	R
Salicaceae					
Populus deltoides Marsh. FAC. [SS]	—	—	R	—	—
Salix exigua Nutt. OBL. [SDF]	—	I	—	F	—
S. nigra Marsh. FACW+. [SS]	I	F	I	F	O
S. sericea Marsh. OBL. [SS]	—	O	—	O	—
Saxifragaceae					
Penthorum sedoides L. OBL. [SDF]	O	O	O	F	I
Scrophulariaceae					
Agalinis purpurea (L.) Pennell. FACW−. [SGM]	—	O	—	I	—
Gratiola neglecta Torr. OBL. [SDF]	—	—	R	—	—
Leucospora multifida (Michx.) Nutt. OBL. [SDF]	—	—	—	R	—
Lindernia dubia (L.) Pennell. var. anagallidea (Michx.) Cooperrider. OBL. [SDF]	I	O	O	F	I
Mimulus alatus Ait. OBL. [SGM]	O	O	O	O	I
M. ringens L. OBL. [SGM]	R	I	—	F	I
Sparganiaceae					
Sparganium americanum Nutt. OBL. [EM]	—	—	—	—	A
Typhaceae					
Typha angustifolia L. OBL. [EM]	—	—	—	F	—
T. latifolia L. OBL. [EM]	—	A	F	A	F
Urticaceae					
Boehmeria cylindrica (L.) Sw. FACW+. [SDF]	O	F	O	F	I
Laportea canadensis (L.) Wedd. FAC. [SDF]	I	—	I	O	—
Pilea pumila (L.) A. Gray. FACW. [SDF]	O	O	I	F	I

Appendix. Continued.

Taxon. National Wetland Category. [wetland habitat]	Relative abundances				
	USC	LSC	CBR	OFR	RLR
Verbenaceae					
Verbena urticifolia L. FACU. [SGM]	R	O	I	R	—
Vitaceae					
Ampelopsis cordata Michx. FAC+. [SDF]	—	—	—	O	—
Parthenocissus quinquefolia (L.) Planch. FACU. [SDF]	O	I	O	O	O
Vitis vulpina L. FAC. [SDF]	I	O	I	O	I
Totals	114	177	150	214	135

Abbreviations and acronyms used:
Berea College Reservoirs: CBR = Cowbell; LSC = Lower Silver Creek; OFR = Owsley Fork; RLR = Red Lick No. 2; USC = Upper Silver Creek.
National Wetland Categories: FAC = Facultative; FACU = Facultative Upland; FACW = Facultative Wetland; OBL = Obligate; NC = Not Categorized; UPL = Upland; + = Wetter limit of facultative categories; − = Drier limit of facultative categories.
Reservoir Wetland Habitats: EM = Emergent Marsh; SDF = Seasonally Dewatered Mud-sand Flats; SGM = Sedge-grass Meadow; SS = Shrub Swamp; SSG = Shallow Stream with Gravel Bar; VOW = Vegetated Open Water.
Relative abundance: R = Rare; I = Infrequent; O = Occasional; F = Frequent; A = Abundant.

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Natural Terrestrial Vegetation of Boone County, Kentucky: Classification, Ordination, and Description

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ABSTRACT

Seventeen mature forests and one prairie in Boone County, Kentucky, were systematically sampled and subjected to cluster analysis and ordination. Eight vegetation or community types were identified: mixed mesophytic (glacial), mixed mesophytic (steep alluvial), beech, beech-maple, oak-hickory, oak-ash-maple (or western mesophytic), floodplain, and prairie. Environmentally, the vegetational patterns observed in the county appear to follow moisture and topographic/soils gradients. Also, differences in glacial and edaphic history appear to strongly influence tree species community patterns.

INTRODUCTION

Geologically and edaphically, Boone County is unlike most of Kentucky. Although bedrock consists of limestone and calcareous shale of Ordovician age, it is the presence of outwash deposits of Nebraskan, Kansan, and Illinoian glaciations plus a loess cover of Wisconsin age (Ray 1974) that largely accounts for these differences (Figure 1). These deposits cover ca. half of the county's 64,578 hectares. Locally, the glacial deposits vary in thickness or are absent where they have been removed by erosion. Soils in the county are nearly equally divided between those of residual origin or those of transported origin—glacial, loessial, alluvial, and colluvial (Weisenberger et al. 1973) (Table 1).

Historically, nine natural areas were listed for Kentucky (Middleton et al. 1926) in *Naturalist's Guide to the Americas*; the two in Boone County were areas with glacial deposits. Later, Keith (1968) attempted to characterize the vegetation on some of the glacial deposits in the county. Held and Winstead (1976) reported on a forest on pre-Illinoian deposits, and Bryant (1978) analyzed a forest on Kansan outwash deposits there. Bryant (1981a) described forests of the unglaciated Eden Shale Belt, which covers much of the southern half of the county, and also a small prairie outlier on a Kansan deposit (Bryant 1981b). At present, ca. 38% of the land area of Boone County is forested (ERMC 2002). A majority of the existing woodland is composed of young successional stages ~50 years in age; however, 11% and 2% of the land area is in medium to large-crown canopy cover, respectively (ERMC 2002).

The climate of Boone County is temperate and humid. The average annual temperature is about 12.2°C, and the average precipitation is about 101.6 cm per year (Elam 1973). There are no regular wet or dry seasons; precipitation is fairly well distributed throughout the year (Elam 1973).

For over 30 years we have been systematically sampling the natural vegetation in Boone County, especially those areas with large crown cover (ERMC 2002). The objectives of this paper were (1) to classify the natural vegetation of the county, (2) to compare vegetation-environmental relationships, especially the influence of soils and glacial history on vegetation, and (3) to document the vegetation in a portion of the Greater Cincinnati area (Boone County) that is experiencing a rapidly

Figure 1. A glacial map of Boone County, Kentucky, and the Greater Cincinnati region showing the extent of drift deposits (after Ray 1974).

increasing human population and subsequent changes in land use.

METHODS AND MATERIALS

In general, trees at all sites were sampled in 0.04 ha circular plots spaced at 30 m intervals along line transects throughout each forest. Two stands were sampled using plotless methods, and the prairie was sampled in 1 m × 1 m plots (Bryant 1981b). We sampled 17 mature forests plus one prairie site from across Boone County; however, one of the forest sites was located in adjacent Kenton County (Figure 2).

Only trees ≥10 cm diameter breast height (dbh) were measured; however, in two stands the minimum dbh was ≥8.9 cm. Although shrubs, seedlings and saplings, and herbs were sampled in most stands, only overstory was considered in the forest analysis for this paper. These data were analyzed to relative frequency (RF), relative density (RD), and relative dominance (RDo), which were then summed to generate an importance value (IV) for each species (Curtis and McIntosh 1951). Density (trees/ha), total basal area (m2/ha), and species diversity (H') were calculated for each forest. Grasses and forbs in the prairie were analyzed to frequency only (Bryant 1981b).

Classification of stands was developed using an unweighted pair-group mean-average (UPGMA) cluster analysis with percent similarity (Kovach 1998). A cutoff value of 60% similarity was used to create the initial cluster groupings (Hinkle 1978). This analysis was performed on untransformed importance value data, which included all species. Stand relationships were summarized using the Bray-Curtis ordination procedure (McCune and Medford 1999).

RESULTS

Forty-four tree species were recorded for Boone County, 38 from our samples plus 6 from Keith (1968). Tree density, basal area, tree species diversity, and tree species richness

Table 1. The percentage (%) of land in Boone County, Kentucky with residual and transported soils. The total percentage does not equal 100 since only soil types for sampling sites are listed. Also the range of slope for each soil type is included.

	%	Slope
Residual soils		
Eden silty clay loam	19.0	12–35
Cynthiana flaggy silty clay loam	8.8	12–50
Faywood silty clay loam	13.9	2–20
Total	41.7	
Transported soils		
Glacial (loess over glacial till)		
Rossmoyne silt loam (fragipan)	23.1	0–12
Jessup silt loam (no fragipan)	14.5	2–20
Loess (loess over residuum)		
Nicholson silt loam	7.9	0–12
Alluvial land, steep	1.4	—
Lakin loamy fine sand	0.6	0–12
Alluvium		
Lindside silt loam	0.9	0–4
Nolin silt loam	1.1	0–4
Huntington silt loam	1.2	0–4
Total	50.7	

for each stand are shown in Table 2. Based on cluster analysis, we were able to recognize three vegetation types: (1) mixed mesophytic (glacial), (2) oak-hickory, and (3) oak-ash-maple (Figure 3). All five of the mixed mesophytic (glacial) stands clustered together at >60% similarity as did two oak-hickory stands and two oak-ash-maple stands. Other vegetation types apparently were represented by only one stand each and did not show clustering.

In an attempt to determine vegetation-environmental relations, all stands were ordinated. Axis 1 of the ordination appeared to be a moisture gradient with the prairie at the drier end and the floodplain forest at the wetter end (Figure 4). The majority of stands tended to group near the middle of the moisture gradient, perhaps reflecting the mesic nature of the county. Axis 2 was either a topographic or soil gradient, but may be a combination of the two. The beech forest was on a flat upland site with Jessup soil, the mixed mesophytic (steep alluvial) site was in a ravine with sandy soils of Illinoian or glaciofluviatile origin (Ray 1974), and the beech-maple site was gently rolling with Rossmoyne soil, which consists of Wisconsin loess over glacial till (Weisenberger et al. 1973). All mixed mesophytic (glacial) sites were in highly dissected areas of Kansan outwash and Jessup soils. The oak-hickory sites were on gentle to moderate slopes with Faywood and/or Eden silt loams; the oak-ash-maple sites were on steep slopes with Cynthiana silt loams. The latter three soil types are residual.

DESCRIPTION OF VEGETATION

Mixed mesophytic forests were of two types, those on Kansan glacial deposits and others on steep alluvium. The mixed mesophytic glacial sites (BC, BN, BP, MU, YP; codes for sites are explained in Figure 2) were on highly dissected outwash deposits of Kansan age. Seeps and springs were characteristic of these sites. Dominant tree species included sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), beech (*Fagus grandifolia*), basswood (*Tilia americana*), northern red oak (*Quercus rubra*), bitternut hickory (*Carya cordiformis*), and tulip poplar (*Liriodendron tulipifera*). Tree species diversity (H′) ranged from 2.6 to 3.5 (Table 2) and these sites are floristically rich (Bryant 1978). Tree density ranged from 222 to 499 trees/ha, and basal areas fit the >25 m^2/ha proposed by Martin (1992) for mature mixed mesophytic forests.

Mixed mesophytic (steep alluvium) forests are found in ravines (SC) with steep alluvial soils. These sandy soils are found within the narrow band of Illinoian glacial deposits near the Ohio River in the western portion of the county (Figure 1). Tulip poplar was the dominant species at this site and also at a sandy hummock site (HS); however, vegetation at that site tended to ordinate more closely to beech-maple than to the mixed mesophytic (glacial) stands.

Oak-hickory forests (DU, WS) were associated with the droughty residual Faywood or Eden soils, although other soils, e.g., Nicholson, were of minor importance. Those stands were located on side slopes and narrow ridges, especially within the Eden Shale Belt portion of Boone County. White oak (*Quercus alba*), northern red oak, and shagbark hickory (*Carya ovata*) were canopy dominants. On such sites sugar maple was primarily a subcanopy member (Bryant 1981a). Other stands (LW, MM, DW) showed similarities to oak-hickory,

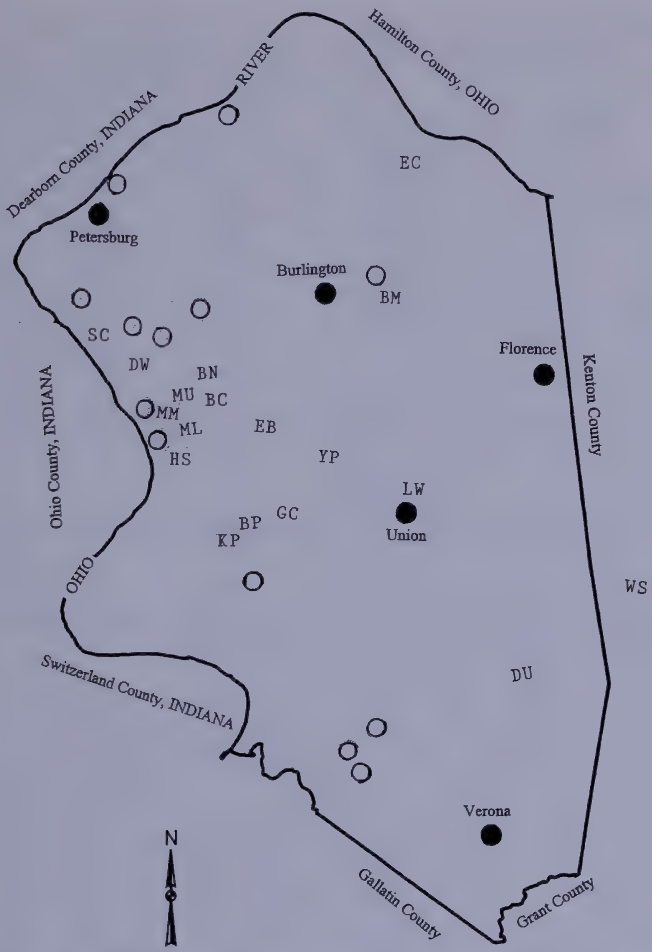

Figure 2. Location of sampling sites in Boone County, Kentucky. Site abbreviations: BC, Boone County Cliffs Nature Preserve; BM, Beech-Maple; BN, Boone County Cliffs Addition; BP, Bald Point; DU, Durr's Woods; DW, Dinsmore Woods; EB, East Bend; EC, Elijah Creek; GC, Gunpowder Creek; HS, Hummock Site; KP, Kansan Prairie; LW, Luebber's Woods; ML, Middle Creek Floodplain; MM, Middle Creek Middle Slope; MU, Middle Creek Upper slope; SC, Steep Creek; WS, Woodland-Scott (located in adjacent Kenton County); and YP, Young Property. Open circles represent Keith's (1968) sample sites; closed circles show location of some towns in Boone County.

but other oak species, not white oak, were of greater importance.

Oak-ash-maple communities (EC, GC. DW) occupied moderate to steep slopes, especially where erosion had removed much or all of the former till deposits. Cynthiana silt loam was the principal soil type and the forest dominants were oaks (*Quercus* spp.), white ash, and sugar maple. Because of compositional similarities, it was often difficult to distinguish oak-hickory and oak-ash-maple stands.

Beech-maple (BM) forests were most prominent on the level to gently rolling uplands with Rossmoyne soils. These loessial soils are underlain by a fragipan that may hinder drainage. Beech (EB) forests were also found on gently rolling uplands, but there Jessup soil is most prominent. Both forest types had low tree densities, but high basal areas (Table 2).

Floodplain forests were dominated by boxelder (*Acer negundo*), cottonwood (*Populus deltoids*), and silver maple (*A. saccharinum*). These forests are located on wide creek banks and Ohio River backwater sites. The alluvial

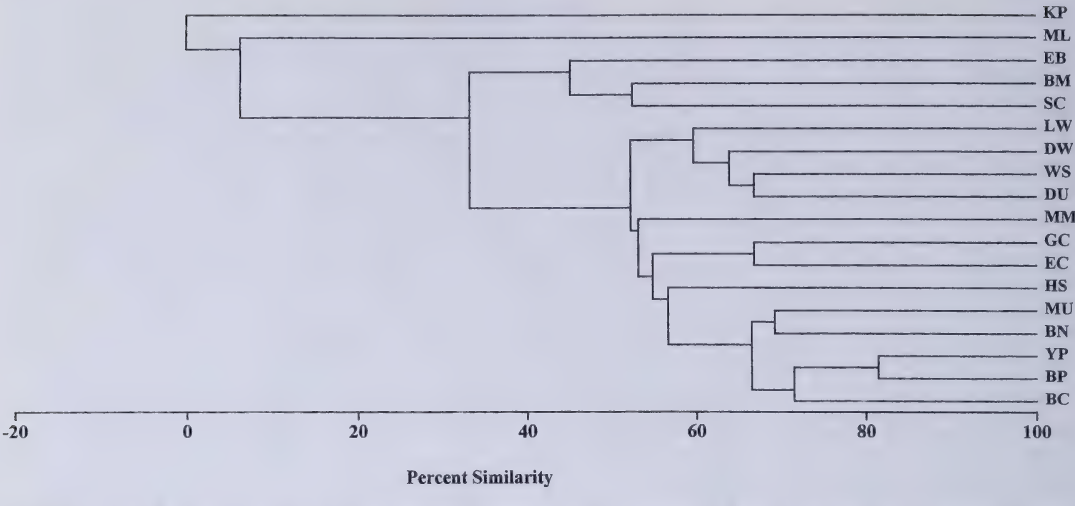

Figure 3. Dendrogram based on cluster analysis, showing the relationship of the sites sampled in Boone County, Kentucky.

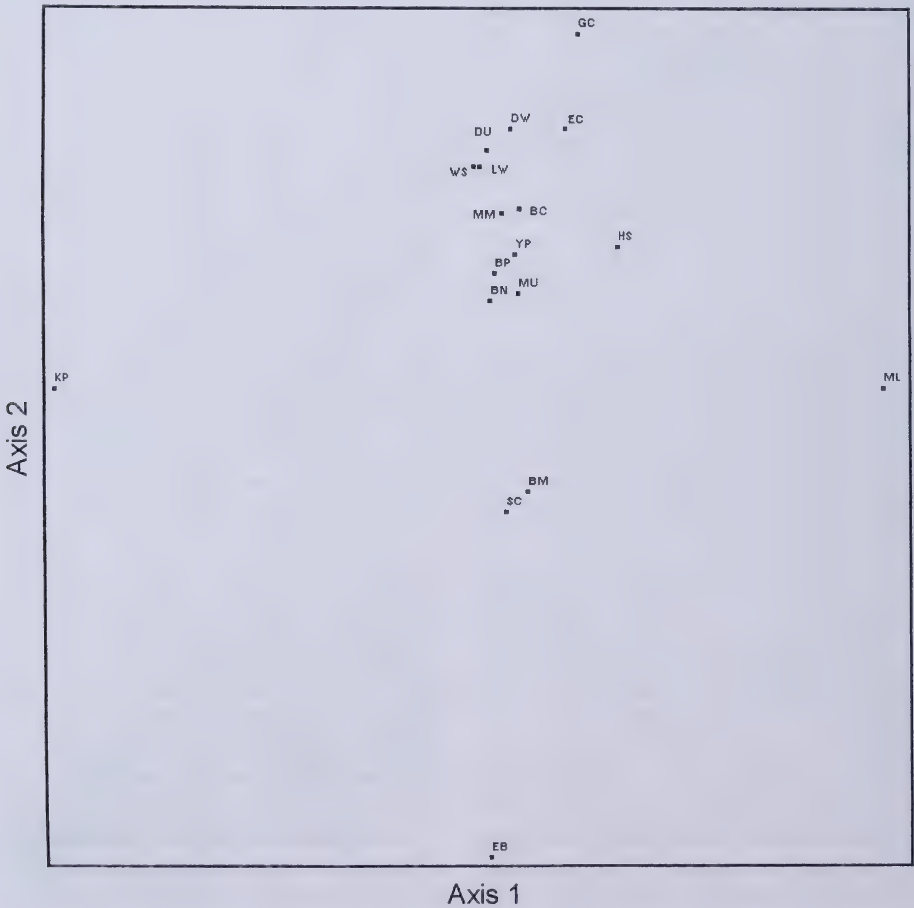

Figure 4. Ordination of the 18 sites sampled in Boone County. Axis 1 is a moisture gradient; axis 2, a topographic/soils gradient.

Table 2. Tree density (trees/ha), basal area (m^2/ha), species diversity (H′), and the number of tree species for forests and other environs in Boone County, Kentucky. Site abbreviations as in Figure 2.

Forest site	Trees/ha	m^2/ha	H′	Species richness (number of tree species)
BC	499	27.8	3.2	25
BN	390	28.3	2.6	18
BP	410	38.4	3.5	20
MU	262	24.7	2.1	12
YP	222	27.1	3.2	23
SC	302	46.0	3.7	20
HS	296	36.2	2.8	16
BM	233	32.6	3.0	18
EB	126	35.4	2.0	8
ML	538	26.7	0.9	9
DU	470	23.1	2.3	17
WS	343	31.4	2.1	13
LW	347	26.6	1.4	11
MM	314	21.5	3.0	17
DW	334	28.1	3.2	22
EC	328	22.4	3.0	14
GC	457	29.6	2.8	16
KP	0	0	0	0

soil complex included Lindside, Nolin, and Huntington silt loams. Species diversity at the wet end of the moisture gradient was lower than for mesic and dry-mesic sites.

Prairie communities (KP) were rare; occurring as small isolated patches on exposed glacial outcrops or upland sand deposits (Bryant 1981b). Little blustem (*Schizachyrium scoparium*), side-oats grama (*Bouteloua curtipendula*), and Indian grass (*Sorghastrum nutans*) were characteristic of these drier sites although a number of forbs were relatively abundant. Seedlings of eastern redcedar (*Juniperus virginiana*) appeared to be slowly invading the prairie.

DISCUSSION

There have been few recent reports or descriptions of countywide (e.g., Campbell and Grubbs [1992] for Hopkins County) or regional vegetation (e.g., Bryant and Held [2001] for the Jackson Purchase Region) in Kentucky. However, Bryant and Held (2004) recently detailed the vegetation-environment patterns in Hamilton County, Ohio. Geologically, Boone and Hamilton counties have experienced similar glacial events; phytogeographically, these two counties are located in the northern portion of Braun's (1950) Western Mesophytic Forest Region but near the junction of her Mixed Mesophytic, Oak-Hickory, and Beech-Maple Forest Regions.

The structure and composition of vegetation are determined by a number of factors including past disturbances, successional processes, and individualistic responses of species to environmental constraints (Cole and Ware 1997). A number of species respond similarily to environmental gradients and thus sort out as communities or vegetation types.

The beech-maple and beech forests that we identified may represent remnants of forest types that formerly were more widely distributed on Rossmoyne soils in Boone and adjacent Kenton counties, Kentucky (Keith 1968). Beech-maple forests were reported on soils of mixed glacial origins in Hamilton County, Ohio (Bryant 1987; Bryant and Held 2004) and were found on Illinoian deposits well south of the Wisconsin glacial border in Indiana (Lindsey et al. 1965). Braun (1950) and Vankat et al. (1975) considered the terminus of the Wisconsin advance in southern Ohio to be the southern extent of beech-maple forests.

Bryant and Held (2004) reported two types of mixed mesophytic forests in Hamilton County, one with tulip poplar and one without. We also found two types of mixed mesophytic forests in Boone County—one on highly dissected Kansan outwash deposits (Bryant 1978) and one on steep alluvium. Although Keith (1968) considered tulip poplar to be scarce in Boone County, it was common in both mesophytic types and was a dominant on the steep alluvium. In southern Ohio, Forsyth (1970) noted that mixed mesophytic forests containing buckeye, beech, white basswood, and tulip tree appeared to correlate with the occurrence of Kansan drift. A great variety of moisture conditions exists in these deep, steep-sided valleys (Forsyth 1970) and supports a mixed mesophytic association.

We found the floodplain forests in Boone County to be compositionally similar to those in Hamilton County. Boxelder, cottonwood, and silver maple, along with green ash (*F. pennsylvanica*) and various willows (*Salix* spp.), vary in importance in different parts of the floodplains.

Oak-hickory forests were found primarily on steep slopes and ridges with droughty residual soils. Keith (1968) speculated that white oak had been more important in the past than at

present. In the Eden Shale Belt, white oak is generally more important in stands where disturbance has been minimal (Bryant 1981a), but its importance was reduced on previously logged sites. This may support Keith's (1968) speculation; however, slope aspect may influence the occurrence of white oak. On less exposed aspects, more mesic species are of greater importance.

Oak-ash-maple stands were most commonly found on steep slopes in those areas of the county where erosion had removed the former glacial cover. In Hamilton County, Ohio, Bryant and Held (2004) referred to this forest type as western mesophytic after Gordon's (1966, 1969) recognition of western mesophytic forests in southern Ohio.

Prairies, although extremely small in areal extent, add an important component to the county's vegetational diversity. These prairie remnants have been maintained by a complex of factors including periodic disturbances (Bryant 1981b).

SUMMARY

The broad vegetational development shown for Boone County might not be expected on a local level (i.e., one county); however, geologic and edaphic diversity underlie and promote biological diversity here. In adjacent Hamilton County, Ohio, Bryant and Held (2004) found forest types to sort out along moisture and topographic gradients. We found a similar pattern in Boone County but perhaps with a stronger influence from soils, especially in relation to glacial and postglacial history. A mosaic of unlike climaxes is characteristic of the Western Mesophytic Forest Region (Braun 1950); we, too, found a mosaic of vegetation types. Braun (1950) considered the Western Mesophytic Forest Region to represent a tension zone where the compensating effects of local environments permit unlike climaxes to exist close to one another. We support that tension zone interpretation for Boone County, especially considering its geographic location and glacial-edaphic history.

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Scientists of Kentucky

Christopher Columbus Graham: Kentucky Man of Science

James Duvall
Boone County Public Library, Scheben Branch, 8899 U.S. 42, Union, Kentucky 41091

Christopher Columbus Graham (1784–1885) was born in Kentucky before it became a state. He was considered one of the most interesting and useful citizens in the state long before the time of his death at the age of 100. Though he was a man concerned in public affairs and a self-made man of wealth, he never held a public office. He was a traveler, writer, archaeologist, medical doctor, civic leader, and philanthropist.[1] In the larger historical picture his scientific contributions must be considered negligible, but he participated in the scientific life of the time and might be considered characteristic, as there were very few men then who could be considered professional scientists.

As a woodsman Graham had few equals. When a young man he made about 20 flatboat trips down the Mississippi. He was on the water near New Madrid, Missouri, during the great earthquake of December 1811. He developed such facility with the rifle that he eventually came to be regarded as the best marksman in the world, and he could shoot with unaided vision to the age of 100.[1] The skills he gained at this period of his life were doubtless of value when he joined a company of infantry during the War of 1812.[2] He was wounded, captured, then exchanged, re-entering active service. Later, at Fort Malden, Canada, he was captured by Indians but soon escaped. In 1814 he enlisted again. These were rough times, and not all of the fighting was with the enemy. For example, Lt. Chasteen Scott (1785–1861), under whom he served as a Sergeant, "whipped a rascally Yankee contracter [sic]" on 30 Oct 1814, the day that Graham and the rest of the company were finally discharged from active service.[3]

Graham probably intended to return to his former occupation as a silversmith in which he had been occupied before the war. As he passed through Lexington, Kentucky, on his way back to Harrodsburg, he chanced to meet Dr. Benjamin Winslow Dudley (1785–1870) in Lexington. Dr. Dudley had received his M.D. at the University of Pennsylvania in 1806, and he had just returned from medical studies in England and France in 1814. He was to be particularly known for his success in operating for kidney and bladder stones; he is said to have operated on 225 such cases, all without benefit of anesthesia, losing only three patients. He also performed successful cataract surgery. He opposed the use of bloodletting and was one of the first to sterilize his surgical instruments in boiling water. He is considered the founder of the medical school at Transylvania University in Lexington. Dudley, slightly younger than Graham, immediately offered him a chance to become one of his first students. Graham, just past the age of 30, was virtually uneducated, except in the ways of the woods, and war, and he told Dudley he lacked both the education and money to study at the university. Dudley replied he would keep the offer open till whenever it was wanted.

After the war was over in the spring of 1815, Graham made another trip to New Orleans. There he accepted an offer from James Hull, D.D., rector of Christ Church, to teach school, which he did, just barely keeping ahead of the students. By this means he likely gained the remedial skills he needed for his future college career. An outbreak of yellow fever, so common in New Orleans at that time, forced him to leave the city. He took a ship to New York, but the ship had its own outbreak, and a number of the people on it died; Graham was ill when he arrived. The ship was quarantined, but he hired a skiff, landed in Virginia, and walked back to Kentucky by way of the Cumberland Gap. He then accepted the offer to study with Dr. Dudley, a turning point in his life.

In 1810 Transylvania University was one of six medical colleges in the nation; by 1820 that number had grown to 26. When Graham be-

Figure 1. Christopher Columbus Graham. From W. B. Allen, *A History of Kentucky*, 1872.

came a student of Dr. Dudley in 1817 it was still one of the best in the country. Graham's quick mind and unusual abilities were put to good use. This was the course of study, according to Wright:

> During this period requirements for the degree of doctor of medicine required a student to take two years of lecture courses, unless he had been a practicing physician for four years, in which case he needed to attend only one year. All candidates had to be twenty-one years or older, write a thesis of not less than twelve or more than forty pages on a designated medical subject, and pass two examinations, one before the faculty and one before the president and trustees. The curriculum covered the areas of anatomy and surgery, theory and practice of medicine, *materia medica* and medical botany, obstetrics, chemistry, and the institutes of medicine.[4]

It has been remarked with some justification (though not entirely correctly) that Graham did very little work in the profession in which he was trained.[5] With an associate, Dr. Henry Miller, he practiced medicine in Harrodsburg for five years following his graduation and did at least some medical work for years afterwards. He wrote a medical book in 1866 titled *The True Science of Medicine*, every copy of which seems to have disappeared.[6] He performed but one operation for kidney stones, and that successfully, on a small child.[7] For over 30 years he was proprietor of Harrodsburg Springs, the most famous "watering-place" in Kentucky at that time.[8] Louis Jacob Frazee, M.D. (1819–1905), writing on the medical uses of the mineral waters of the state, said:

> Thirty years ago the Harrodsburg Springs under the management of Dr. Graham, was one of the most popular watering places in Kentucky. The beautiful grounds, and the fine hotel accommodations presenting the most attractive features of the place. The most important ingredient of the water here, is sulphate of magnesia, which renders it aperient, but aside from this it possesses no very decided medicinal virtues. It may be used in torpor of the bowels, especially when accompanied with dyspeptic symptoms, indeed in almost every case where a gentle aperient effect is desired. This has long since been abandoned as a watering place.[9]

No doubt Graham did at least some consultation during that time, which was probably a factor in the popularity of the springs as a place of healing. He had on his staff in the 1840s Dr. E. B. Thomas, a hydrotherapist.[10] The hydrotherapy department had been organized by Dr. Roland S. Houghton of New York, a famous teacher of hydrotherapy.[11] VanArsdall, after a lengthy analysis of the treatment at Harrodsburg Springs, concluded, "It is obvious that Dr. Graham must have practiced a mild sort of psychotherapy in association with the baths and general hygienic measures." [12] In his old age Graham is listed in the census records as a "Retired Physician," which gives credence to the idea that he considered medicine his primary occupation.

Psychology, then called mental philosophy, was an area in which Graham studied that may be considered an offshoot of his medical interests. His *The true science of mind* (1869), is a devastating critique of the current "mental philosophy," and it is also his own attempt at a positive contribution to the field. He wrote:

> My natural turn of mind led me, in early life, to moralize upon all events, and caused a pleasure (no self-creation, take notice) in me to do just as I pleased, which was to read everything I could find upon the subject of mind. With this foundation I commenced my practical study of mind, and having for more than fifty years mixed with all nations and languages of the human family, from the native Indian up, or rather, down, to the snobs, parvenus, and paragons—the fashionable folly of our race—have sought to find where happiness dwells.[13]

Unlike practitioners of the discipline in our day, Graham tended to draw an overt moral from the material he discussed. He related the following incident (reminding us, perhaps, of Pavlov's dog) about his pet, Fidel:

> I here relate a little incident that illustrates two great leading principles: I had a sprightly and interesting puppy, to which the cook often threw egg-shells, thus teaching it to eat eggs; the result being the breaking up of all my setting hens and loss of chickens. All this, however, my fond attachment induced me to put up with; but another branch of education caused its death. Breachy sheep occasionally entering my yard, I set little Fidel after them, and was often greatly amused to see the little creature in full chase, tight and tight after a flock of great sheep; when on its return it would frisk around me; and looking up with innocent laughter seemed to say: "I did what you told me; wasn't it funny?" By and by, however, till I thus lost ten or twelve by its example in learning others to help it, had I courage to take its life, knowing that the fault was not in the dog, but in myself; nor had I myself a heart, or will, to perpetrate

> the deed, but hired another to do it. This illustrates two vital principles: 1st. The force of education for good or evil, even upon the brute; 2d. The necessity of punishing, or taking the life, to prevent disastrous consequences, and to save the lives of many. Parents and friends often think it smart to hear their little ones swear or commit innocent depredations, as I did little Fidel, not thinking it might lead to their destruction.[14]

His criticism of "faculty psychology" was particularly acute. At one time as many as 40 "faculties" were admitted by some authors, and each author had a different list. Graham did not accept that series of errors. In a chapter titled "What is a Faculty?" he began by saying:

> Faculty is a word coined by the manufacturers of shoddy text-books on psychology, mental philosophy, or metaphysics—all meaning the same thing. . . .[15]

The word, he said a little later, conveys a false meaning and is enforced by arbitrary authority.[16] This is similar to current opinion on the subject. T. H. Leahey, in the *Encyclopedia of Psychology,* wrote, "Strictly speaking, faculty psychology died in the 20th century, at least as regards scientific psychology. Under behaviorism, psychologists became skeptical of inferred entities of any sort, including the mind, which therefore had no need of subdivisions."[17] Graham said in the preface to his work, "I shall strive to drive innate ideas, as witches have been done, from the world, and to show that this thing called divine *conscience* is a parasite—an effect—is not a principle—has no separate existence from the prejudice and education of the mind. . . ."[18] His object, as he said, is the application of natural law to man, and this is certainly a scientific objective. He offered a one-line summary of his theory: "*God has endowed us with sensibility, from which arise pleasure and pain, and consequently a desire or will to do or not to do!* thus is resolved in a short sentence, the mighty question, the great enigma of psychology, soul, mind or intellect, all meaning the same thing."[19]

The reason Graham spent so little time and effort in the field of medicine was probably because the medical profession at the time was badly paid, with the exception of some of the large practices of fashionable city doctors. But also he was extremely skeptical about the benefits that medical practitioners could confer on their patients. The medical profession at the time was in disarray, and theories abounded. Before 1850 there were published in this country alone 213 different medical journals, some of them surviving only a few issues.[20] Strictly speaking, there was no science of medicine, and anyone who was interested primarily in science could only look on the discipline (or lack thereof) in despair. This situation, in some fashion or other, extended to many of the allied sciences, such as chemistry. Graham remarked:

> The chemist, though acting upon the necessary laws of science, is as often disappointed in his results from the endless and unseen counteracting influences, as the man well acquainted with human nature is of the anticipations of his results. The physician, in like manner, is constantly perplexed and disappointed in the sequences of his prescriptions; for though calomel be a purgative, and tarter will puke, calomel may vomit, and tarter purge, from some necessary existing, yet unseen, condition of system.[21]

A little further in *The true science of mind* he made an analogy between the medic and the "superficial metaphysician":

> The physician, when ignorant of those occult workings upon his patient, and pressed hard for explanation, treats the case with deep gravity and most learned technicality; such as morbid irritation, normal and abnormal condition of system; loss of sensorial power, accumulated excitibility, revulsion, translation, concatenation, and above all, "*vis medicatrix natura*" is dragged in as the universal panacea of medical ignorance.[22]

He quoted at length a well-known passage from the French physician Francois Magendie (1783–1855), called by one of his own students "the great sceptic." Graham quoted at some length from one of Magendie's famous lectures, which begins "Gentlemen: Medicine is a great humbug."[23] And this seems to mirror what Graham thought of the medicine of his day, that is, he realized it was not a science. Claude Bernard (1813–1878), the student of Magendie mentioned above, was the first to set medicine on the road to becoming an empirical science. Bernard wrote in his most important book, published in Paris in 1865, "We are doubtless far from the time when all medicine will be scientific; but that need not prevent our conceiving it possible. . . ." This may

serve to justify Graham's doubts concerning the scientific nature of medicine at the time.[24]

Graham's contributions to the progress of medicine, at least on the local level, were practical, and it may have been his practical nature that led to his more intrepid exploits in connection with the medical school. The postmortem examination of an Irishmen, killed by one of his fellow countrymen in a quarrel, led to difficulties between Dr. Dudley, the mentor of Graham, and Dr. Daniel Drake (1785–1852), the professor of medical botany and materia medica. Feelings on the controversy ran high enough that Dudley challenged Drake to a duel, something fashionable at the time. Drake declined the honour, but his friend, Dr. Richardson, professor of obstetrics, accepted in his place. This was duly arranged with Graham as the second of Dr. Dudley; Graham even cut the gold buttons off the coat Dudley had bought in France, so as not to provide a target. At the first exchange of fire, Richardson, who missed, received a potentially fatal wound, which was immediately stanched by Dudley, saving his life. From this time the two professors became fast friends.[25]

But what of the Irishman who caused all the trouble in the first place? There was no sense in allowing him to go to waste, and this led to what the newspapers of the time called "The Battle of the Graveyard."[26]

During the period Graham was at Transylvania, the French physicians were the most medically advanced in the world. Pathological medicine was their great contribution to Western medical science: the concept of tracing a disease or illness to a specific organ or part of the body. The introduction of pathology, though then in its infancy, was (for good or ill), the single greatest factor changing the course of medical research in the United States. Pathological medicine logically led to surgery as the means of finding and removing the cause of illness. Dissection, which is necessary to train a good surgeon, required a supply of cadavers, and, in the absence of any legal means of obtaining them, was a major problem for medical science. Graham wrote years later that he had "headed all the resurrecting expeditions."[27] He said, "I was Dudley's favourit [sic] and well I might be, and was his only dependence in procuring subjects, and was his demonstrator, often dissecting all night while others were out on pleasure."[28]

In 1822, very near the time of "The Battle of the Graveyard," the following advertisement appeared in Wooler's *British Gazette*:

> Many hundred dead bodies will be dragged from their wooden coffins this winter, for the anatomical lectures (which have just commenced), the articulators, and for those who deal in the dead. . . . The violation of the sanctity of the grave is said to be needful, for the instruction of the medical pupil, but let each one about to inter a mother, husband, child, or friend, say shall I devote this object of my affection to such a purpose; if not, the only safe coffin is Bridgman's Patent wrought-iron one.

David Burrell, in an interesting paper on the origins of undertaking, from which the preceding quotation was taken, remarks that Cincinnati, with six medical schools in the area, more than any other city in the West, was very concerned about body-snatching. He pointed out that the metallic coffin, intended to keep the body safe, may have actually aided the body-snatchers by keeping their quarry fresh longer. At any rate these intended final resting places were guarded by walls and watchmen (the fence around the graveyard was never intended to keep the occupants in), and even with landmines, torpedoes, and spring guns that exploded when disturbed. As Burrell, who has written several papers on aspects of this topic, says: "Communities literally feared for their dead each time the medical schools began a new session."[29]

Graham said that once his party was pursued near Nicholasville while making their way from a cemetery to their horses, and one of several shots lodged in the subject he was carrying on his back. He wrote concerning the cause of the trouble between his professors,

> We were taking up the Irishman that caused the duel as above named, and again taking [taken] prisoners by an armed guard and we hauled up to the court for trial but there was no law to make the dead private property, so the declaration of the Scriptures, that from dust we came and unto dust we must return let us off by paying one cent damages for taking that much clay or soil.[30]

"The Battle of the Graveyard" led to what must have been an interesting court case. The defense was by a top-notch lawyer, John Jordan Crittenden (1786–1863), who was governor of Kentucky from 1848 to 1850, just re-

signed from the U.S. Senate in March 1819 to return to his private practice in Frankfort. One writer remarked,

> In all likelihood the judicial decision not to order the body's return did not hinge upon any "convincing" quote of Genesis . . . but because the right to sue for something must inhere in a preceding right to control or own.[31]

This is probably a misunderstanding of the situation. The lawyer and the judge were obviously aware of Blackstone's *Commentaries* (Book 4, Chapter 17) "Of Offences Against Private Property," which stated that among the Romans the stealing of a corpse (though indecent) was no felony unless one stole some of the grave clothes with it. Genesis was readily admissible into any courtroom in Kentucky at this time, and the passage ". . . dust thou art, and unto dust thou shalt return" (Gen. 3: 19) was not irrelevant; it provided the means of settling the case while technically letting the plaintiffs win: Dust was cheap.[32]

Graham's scientific interests extended far beyond the field of medicine. Among the papers of Henry Clay (1777–1852) is a letter from Dr. Graham written to Clay in 1825 when Clay was Secretary of State under President John Quincy Adams (1767–1848). Graham, who had already traveled fairly extensively in the West and Mexico, told Clay he was interested in any kind of government mission that would pay him expenses for travel. He hoped in this manner to serve his country as well as further his scientific interests:

> I am fond of the sciences of Natural History and mineralogy, which traveling so much extends. My cabinet is already respectable, and I wish to enlarge it.[33]

In 1805 the entire mineralogical collection at Yale University fit in a single candle box. It, like the somewhat larger collection at Harvard, was but recently acquired. In 1810 a European collection of about 10,000 specimens was on loan for public exhibit at Yale. This was finally purchased by the college in 1825, the year in which Graham already had a "respectable" collection. A public subscription for the Yale collection was made, and the purchase price was $20,000. Scientific collections of materials all over the country were scanty; for example, Harvard's herbarium was insignificant until the 1840s.[34] The work of private collectors was important since the biological and earth sciences had not yet completed collecting, describing, and classifying materials.[35]

In the letter referred to above Graham reminded Clay they had met several times and had become acquainted at Transylvania University. (Clay was a trustee of that institution.) Graham also mentioned that he had become proprietor of Harrodsburg Springs, inviting Clay to visit next time he was in the area. He discussed some political matters relating to Andrew Jackson, always of interest to Clay. Then, in the last paragraph of the letter, he made the following statement:

> I should be pleased to know the probability of Mr. Rafinesque's success in his Banking schemes, as he has flooded me with letters, appointing me *sole agent* in all his operations. I know him to be so visionary, that I have given the subject but little attention.[36]

There is a likely connection between Graham's interest in natural history and his acquaintance with Constantine S. Rafinesque (1783–1840). It may have been Graham's practical business instincts that led Rafinesque to fix upon him as suitable to advance his scheme of divisible stock coupons. Graham's consideration of Rafinesque is in keeping with the general opinion of him at the time. Rafinesque related that he visited Graham in the course of his travels in Kentucky:

> I visited again the small cabinet of Maj. Thompson at Shawnee Spring near Harrodsburg, that of Shells of Dr. Graham at Harrodsburg, and that of curiosities by Mrs. Mc'Dowell at Danville. I went on horseback with Dr. Graham to survey ancient monuments on Salt R. where we dug fossil teeth.[37]

Dr. Graham was a life-long naturalist. We have some of his descriptions of geological formations, one of which is the "Devil's Pulpit" along the Kentucky River in Nelson County.[38] Frederick Hall, M.D. (1780–1843), who visited Graham's Springs in June 1837, wrote,

> I cannot help saying, that our polite landlord is admirably qualified for the management of this immense establishment. Doctor Graham is a well-informed physician, gentlemanly in his deportment, and exceedingly accommodating to all his boarders. His many kind attentions have brought me under lasting obligations to him. We have taken several long walks together. To-day he made me acquainted with an interesting locality of the sulphate of barytes. It is about two miles south from his house. The substance

forms a vein, in secondary limestone rock, which is nearly perpendicular to the horizon, varying in thickness from one to eight inches, and extending, according to Dr. G. at least, twenty miles in length. The mineral is white, and its structure laminated.[39]

Graham explored caves and was an inveterate collector of fossils and other natural artifacts; in 1872 the large cabinet he collected was estimated to be worth $250,000. His material was donated to the Museum of the Kentucky Free Public Library (Louisville) at that time, and he began to assemble another collection. In his archaeological work at Big Bone Lick, Graham was not content merely to collect bones, but he formed a theory concerning the geology of the region, which I will not cover here; he described the valley and explained why it was sinking. Graham did not merely collect, like Prof. Rafinesque, but he also wrote accounts in which he attempted to set forth the significance of what he had found.

Dr. Graham had been a close observer of wildlife from an early age. He once compared himself to John James Audubon (1785–1851), the famous naturalist and painter of birds, with no advantage to the latter:

Audubon was never a closer observer of birds than I have been of all animals; so much so, indeed, that I have learned the language of many of them. I can tell by the voice of birds when they see a serpent as well as if I were to see it myself.[40]

In the early part of the 19th century the biological and earth sciences were still in the process of collecting, describing, and classifying materials. Rafinesque, often exuberant and careless in his methods, was among the first scientists of note in the West to engage in this necessary task on such a large scale. His methods and manner left him open to criticism, so that he did not always get credit for what he actually accomplished, but part of his legacy in the West—Kentucky at that time—was his students. He is said to have been the first (if not *the* first, one of the early few) to actually use specimens in the classroom. He was an indefatigable traveler, collector, and writer. He attempted to found a botanical garden in Lexington, but the project came to naught. A professor of Rafinesque's caliber would have left a profound mark on a student such as Graham. Even if Graham never sat in his formal lectures (though it is likely that he did), Transylvania University was too small for such an influence to be unfelt. Graham, with his interest in nature and science, whatever reservations he might have had about Rafinesque's "visionary" tendencies, would have gravitated to a teacher with such a background and knowledge.[41]

Graham's work in geology and archaeology may have been his most visible contribution to scientific work in Kentucky. In 1871 the Public Library of Kentucky was incorporated by the Legislature. Former Gov. Thomas Bramlette (1817–1875), Graham's son-in-law, and Reuben T. Durrett (1824–1913), of the Kentucky Historical Society, who also married one of Graham's daughters, were the chief organizers. They raised over $400,000 (by means of a lottery, though the word "lottery" was carefully avoided) to endow it. The next year a building for the library was purchased for $210,000. There were placed in it 40,000 volumes, and a Museum collection of 250,000 "specimens." Graham was named curator of the museum and was the chief donor of the artifacts. It was in this capacity that he conducted the excavations at Big Bone Lick. The Library itself soon went bankrupt, amid charges of fraud and mismanagement.[42] Its effects went to the Polytechnic Society of Kentucky, and later to the Louisville Free Public Library. It may not be possible to even trace the materials at this point, since the Louisville Free Public Library later gave away all the artifacts to museums across the country, apparently without documenting where anything went.[43] As late as 1884 Graham still had materials to sell and donate, as he wrote to John P. Knox (1844–1903), the state geologist.[44]

Elsewhere I have published Graham's account of his excavation at Big Bone Lick, Boone County, Kentucky; only a brief summary is given here.[45] For 30 days he dug with 10 men from the area who were paid one dollar a day. Graham was then 93 years old. He wrote not only about his own excavation but also provided a glimpse of prior digs in the area. He was fortunate to have as one of his workmen Thomas Rich (1808–1883), who had dug there on various excavations for years. Nathaniel S. Shaler (1841–1906), mentioned here, was professor of geology at Harvard University. He excavated at Big Bone Lick in 1863. Graham said in the article:

> In the year 1833 Thomas Rich, as above named, who dug for me, was foreman in excavating for an agent of New York, and exhumed a gigantic skeleton, twenty-two feet long and eleven feet high, with tusks twelve feet long, all of which are now in the Kentucky Department of the British Museum, in London. Mr. Rich told me that he also disinterred, during the same digging, the skeleton of an elk whose horns were seven feet long, since which he has been digging for various parties, among whom was Mr. Shaler, our present geologist of Kentucky, but never found an entire skeleton of any kind; and I to save money to those who may wish to search, say that I am satisfied there will never be another found. I obtained petrified horns of both deer and elk, but not very large.

There are several important things about Graham's investigation. The most significant seems to be the following discovery, which he owed to Mr. Rich and the local workers:

> In my excavations we often came to piles of what I thought were collections of yellow, soft sand; but seeing the old diggers rubbing it between their hands till warm, and smelling it with a grin and leer of the eye that said to me you are green, I found it to be decayed flesh that still had the odor, as dogs around the pit would smell and scratch into it.

I am not aware that any other excavator mentions the preserved remains of the flesh of these creatures. If this is true, and I see no reason to doubt that it is, it would certainly be relevant in any inquiry concerning the age of the beasts whose bones have created so much interest. If conditions have continued to preserve this flesh in a state similar to the present, it might even be possible for archaeologists to acquire DNA samples from it, which would doubtless extend our knowledge on the whole subject of the animals inhabiting the Lick in prehistoric times.

One of Graham's other discoveries of more local historical interest at Big Bone Lick is his finding the remains of the salt-boiling works, which ceased about 1812. He found them six feet below the surface, and he offered an explanation as to why the ground should be littered with preserved bones (that is, hard and undecayed) when the Lick was discovered, but the bone-bed should be so far below the surface, 10 to 12 feet, in 1876. So far as finding artifacts is concerned, the excavation could probably be called a success, as Graham reported that he "brought off seven barrels of bones, a number of buffalo heads, and both mammoth and mastodon molars but found no very large bones or tusks (but had nine feet of a 14-foot tusk given me by Mr. McLaughlin, proprietor [of the hotel]), and left upon the ground a cart load of bones of various animals."[46] But it was significant in other ways, and it speaks well for Graham as a scientist that he was concerned to publish an account of his findings rather than merely carry away bones, as was the habit of so many of the others who sought bones at the Lick.

Dr. Graham was in communication with a number of eminent scientists. He wrote his account of the excavations at Big Bone Lick at the request of Prof. Fredric W. Putnam (1839–1915) of Harvard University. Professor Putnam became curator of the Peabody Museum of American Archaeology there in 1875, a year prior to the work at Big Bone; he held the position until 1909. Appointed professor of archaeology and ethnology at Harvard in 1886, he is considered one of the first anthropologists to work in the United States. In 1874 he served as the assistant to the Kentucky Geological Survey under Dr. Shaler, and it was likely that he met Dr. Graham at this time.

Graham also corresponded with Charles Darwin (1809–1882). In a four-page letter dated 30 Jan 1877, he informed Darwin that he had defended him, John Tyndall (1820–1893), and others against the attacks of a clergyman.[47] It seems Graham also sent him a copy of the article on Big Bone Lick, which had been published in the *Courier-Journal* (Louisville) on 29 Jan 1877, and this was probably the real reason for the letter. Darwin is said to have commented favourably on the article and to have passed it on to Thomas Henry Huxley (1825–1895) and others, who made similar comments on it. There are two short letters Graham wrote in 1880 (28 March and 17 April) in which he informed Darwin that his earlier letter had been framed and was to be placed in the Kentucky statehouse. No doubt this was to be in the fireproof rooms that had been given the Kentucky Historical Society, of which Graham was a charter member.

In 1820 Graham married Theressa Sutton, daughter of the proprietor of the Harrodsburg Springs, and soon owned the resort himself. Here is how Frederick Hall, himself a medical doctor, described it:

> Our stage drove directly to the hotel of Doctor Graham, the proprietor of one of the springs, and of the principal public house in the village. I soon betook myself to the healing fountain. Its waters, I found, contain sulphuretted hydrogen, carbonic acid—much less abundant, however, than at the Congress, or Round Rock Spring, of Saratoga—together with the sulphates of soda, lime and magnesia. They send forth a disagreeable odor, and yet the water is not offensive to the taste. I drank two tumblers of it with a good *goût.* Its effect is cathartic. It operates speedily on the bowels, and produces a cleansing, salutary result. There are other springs in the neighborhood, comprising different ingredients, and producing different effects, and which are known by the appellations of Salt Spring, Chalybeate Spring, and Vitriol Spring.—They do things here on a broad scale. The Spring-house, and hotel, in which I am lodged, is sufficiently capacious to accommodate seven hundred boarders, and it is, Doctor G. assures me, filled during the watering season, to overflowing. It covers more ground, he remarks, than any other public house in the United States.[48]

In the next year or so the hotel was expanded to accommodate 1000 people.

Marriage and settling into a medical practice did not end Dr. Graham's adventures. In 1822 he was in Mexico City with one of his schoolmates from Transylvania, Stephen A. Austin (1793–1836), who was the cousin of Mrs. Mary Austin Holley (1784–1846), wife of the president of the university. There he met Gen. James Wilkinson (1757–1825), famous in Kentucky for his part in the Burr Conspiracy.[49] Graham is supposed to have smuggled out of the city, in a pair of worn out old shoes, the constitution Wilkinson wrote for the new government of Mexico. In 1826 Graham bought the Greenville Springs near Harrodsburg, though he did not operate it as a resort; he gave part of the land to found two educational establishments. A letter published in the *Kentucky Reporter* (Lexington) from General Andrew Jackson reported that the General, not quite at the height of his fame, was contemplating visiting Harrodsburg Springs on account of his wife's health but changed his mind when she recovered.[50]

In 1832 Graham bought a lead interest at Galena, Illinois. There he made acquaintance with another fellow Kentuckian and alumnus of Transylvania, Lt. Jefferson Davis (1808–1889), just before the Black Hawk War began.[51] After the war was over in spring 1833 he returned Black Hawk and his family to their home in Iowa aboard his steamboat.[52]

In the years following, Graham was absorbed in developing Harrodsburg Springs and improving the roads in the area, which helped his business. He is said to have financed many improvements to the public roads himself. Graham claimed to have put $300,000 into the resort at Harrodsburg Springs, which by then was often referred to as Graham's Springs. In 1853 he sold the resort and the 203 acres on which it stood. It was bought for $100,000, for use as a military asylum by the United States government.

Graham and his oldest son, Montrose, were on the Survey of the Southern Atlantic and Pacific Railroad, under Col. Gray in 1852. Such surveys were important in the development of science in this country.[53] Graham served as the surgeon on this expedition. There were a number of incidents, as when the party was captured by the Apaches and ransomed. Graham soon found himself at odds with Gray and broke off for his own trip into Mexico. He was looking for a geological formation of which he had heard, a vast mountain of iron worth a fortune, though never found. Mexico was in a civil war, and Graham's party barely escaped with their lives. When they got to the Pacific they took a boat for San Francisco, but it was unseaworthy, and they were at sea for 69 days with little food or water.

A year later Graham purchased Sublimity Springs and 1500 acres on the Rockcastle River for $20,000. He built a hotel and a flour-and-saw mill and greatly improved the property, but the days of the watering-places had reached their zenith, and Sublimity never attained the stature of Harrodsburg Springs, which burnt a few years later. In 1858 the Springs was operated for Graham by Campbell and Kromp. Lodging was $5 per week, and $3 a week for horses. He published a book in New York in 1859 titled *Man from his cradle to his grave.* It was a 451-page work on psychological subjects, but I have not been able to get access to it. In the same year his wife, Theressa, died.

Graham devoted much of his time in this period to writing. In 1861, when he was 76, Graham married Miss Columbia Buford, age 22, and moved to Crab Orchard, Kentucky. She died in 1864, in his 80th year, soon after

the birth of their son, the seventh of his children, and the only one to follow their father into the medical profession. In 1862 Graham was illegally arrested by Union soldiers, one of whom he severely wounded, as a Confederate sympathizer. He was imprisoned for a week and then released. He published *The true science of medicine* in 1866 and The *true philosophy of mind* in 1869.[54] The next year began his association with the Public Library of Kentucky. Richard H. Collins (1824–1889) published the two-volume *History of Kentucky* in 1874 and acknowledged the contributions of Dr. Graham to his research. From this point until his death in 1885 Graham became more and more involved in the history of Kentucky, writing letters to people such as Lyman C. Draper (1815–1891), one of the foremost collectors of manuscripts and other materials for the history of the West.[55] In addition Graham wrote a series of articles about the early pioneers of Kentucky, which appeared in the *Louisville Monthly Magazine* (1879).

In 1880 Graham became a charter member of the Kentucky Historical Society; indeed, it is probably significant that his name is the first on the list in the charter granted by the state legislature. When he was 90 he still went on frequent expeditions for geological specimens, which would have included the excavation at Big Bone Lick. Col. E. C. Walton, the 50-year editor of the Stanford *Interior Journal*, remembered that Graham, at age 97, walked the 10 miles from Crab Orchard to Stanford to eat a birthday breakfast with a friend.[56] For his 100th birthday his friends in Louisville held a centennial celebration at the Louisville Hotel, consisting of a huge pioneer dinner and many, many toasts, all of which was written up and occupied most of the front page of the *Courier-Journal* along with a wood-cut showing Graham as a venerable-looking gentleman with long white flowing hair. This event was felt by many of those present, and his acquaintance, to be the passing of an age, since he was the last living link with the early past of Kentucky, and he had been acquainted with most of the pioneers of note.[57]

Upon the grounds of the Harrodsburg Springs—the sole remaining relic of the era when the resort flourished under Dr. Graham—is a tombstone marked "Unknown." It is not the stone of Graham. At his death on 3 Feb 1885 he was buried in the cemetery in Danville, not far from the place of the pioneer fort in which he was born on 10 Oct 1784. It is perhaps fitting that the stone of one who began his scientific career robbing graves should have been stolen.[58] The stone on the grounds at the Springs marks the grave of a society girl of the south who visited and, after dancing all night in the fashionable ballroom, fell dead in the morning.[59] This romantic incident seized the public imagination, and passed into Kentucky legend, forever to be associated with Graham's Springs. It may serve to remind us that there are many things we would like to know about Graham and the people of this era, but also there is much we owe them.[61] The past, though vanished, still lives in the collective memory through the writing and reading of history and offers us insight into the present.

ENDNOTES

1. Thomas D. Clark, *The Kentucky* (1942; rpt. Lexington: University Press of Kentucky, 1992), p. 223. A $10,000 was reward offered and advertised in Europe and America to anyone who could out-shoot Graham.
2. G. Glenn Clift, *Notes on Kentucky veterans of the War of 1812.* (Anchorage, Kentucky: Borderland Books, 1964), p. 342.
3. *Kentucky Soldiers of The War of 1812.* Report of the Adjutant General of the State of Kentucky. (Frankfort: E. Polk Johnson, 1891). He is reported to have served from 1 Jan 1814 till 31 Oct 1814. There is some indication that this was his second period of service in that war.
4. John D. Wright, *Transylvania, Tutor To the West.* (Lexington: Transylvania University, 1975; rpt. University Press of Kentucky, 1980), p. 84.
5. Russel Blaine Nye, *Society and Culture in America, 1830–1860.* (New York: Harper & Row, 1974), p. 342 remarks that in this period nearly all physicians were general practioners, and the income was not high. As late as 1873 there were but 149 hospitals in the entire country, and a country doctor might make $3000 a year.
6. Extensive searches have been made for this work. I would like to thank my colleagues Jinny Ussel and Michelle Foster, of the Boone County Public Library, Union, Kentucky, for doing an exhaustive internet search for this book, and Maggie Heran of the Lloyd Library, Cincinnati, for searching their collection of medical texts for the same. Altsheler wrote of this book in 1933: "Enquiry was made in a number of libraries, including the larger libraries in Louisville and the Library of Congress, but no copy was located.

The Filson Club will be glad to have its attention called to the whereabouts of any copy." p. 87, n. 19.

7. This is reported in the letters published by Prof. Hamon cited below.
8. See Clark, "Graham's Springs," *The Kentucky*, Chapt. 14, p. 220–237, and C. A. VanArsdall, "A Medical History of the Harrodsburg Springs." *Bulletin of the History of Medicine*, Vol. 23, No. 4 (1949): 387–418.
9. L. J. Frazee, M.D. "The Mineral Waters of Ky." A Paper Read before the Kentucky State Medical Society April 1872; rpt. from 17th Vol., *Transactions* of the Kentucky State Medical Society. (Louisville: Hart & Mapother, 1872), p. 6; Kentuckiana Digital Library, KYVL. *Aperient* means slightly laxative, and by extension, causing appetite.
10. This is probably the person listed as Bernard Thomas, age 30, along with his wife, in the Graham household, along with many other families who were staying at the hotel, in the 1850 census. He was born in Maine, and his occupation is listed as a physician.
11. VanArsdall, p. 404, 411. The book which caught Dr. Graham's attention is Roland S. Houghton, ed., *Bulwer, Forbes, and Houghton on the Water-Treatment: A Compilation of Papers on the Subject of Hygiene and Rational Hydropathy* (New York: Fowlers and Wells, 1854).
12. *Ibid.*, p. 413.
13. Christopher Columbus Graham, *The True Philosophy of Mind.* (Louisville: J. P. Morton and Co., 1869), p. 61. Graham's book is full of references to Paley and other theologians. Nye, p. 237, remarks that Paley's *Natural Theology* (1802) was "such standard reading for educated Americans that references to him need never be explained."
14. *Ibid.*, p. 91. *Breachy* means "apt to break fences or to break out of pasture."
15. *Ibid.*, p. 148–153.
16. *Ibid.*, p. 149.
17. T. H. Leahey, "Faculty Psychology," in Encyclopedia of Psychology. ed. 2, R. J. Corsini, editor. (New York: Wiley, 1994): II, 6–7.
18. *True Philosophy of Mind*, p. 11.
19. *Ibid.*, p. 12.
20. Nye, p. 355.
21. *True Philosophy of Mind*, p. 28.
22. *Ibid.*, p. 40.
23. Magendie, who was President of the French Academy of Science, wrote:

    Let us no longer wonder at the lamentable want of success which marks our practise, when there is scarcely a sound physiological principle among us. I hesitate not to declare, no matter how sorely I should wound our vanity, that so gross is our ignorance of the real nature of the physiological disorder called disease, that it would perhaps be better to do nothing, and resign the complaint into the hands of Nature, than to act as we are frequently compelled to do, without knowing the why and wherefore of our conduct, at the obvious risk of hastening the end of the patient.

    Gentlemen, medicine is a great humbug. I know it is called a science. Science indeed! It is nothing like science. Doctors are merely empirics when they are not charlatans. We are as ignorant as men can be. Who knows anything in the world about medicine? Gentlemen, you have done me a great honor to come here to attend my lectures, and I must tell you frankly now, in the beginning, that I know nothing in the world about medicine, and I don't know anybody who does know anything about it . . . I repeat, nobody knows anything about medicine. . . .

    We are collecting facts in the right spirit, and I dare say, in a century or so, the accumulation of facts may enable our successors to form a medical science. Who can tell me how to cure the headache, or the gout, or disease of the heart? Nobody. Oh, you tell me the doctors cure people. I grant you people are cured, but how are they cured?

    Gentlemen, Nature does a great deal, imagination a great deal; doctors—devilishly little when they don't do any harm.

24. Claude Bernard, *An Introduction to the Study of Experimental Medicine* (London: Macmillan, 1927; reprint New York: Dover, 1957), p. 214.
25. Christopher Columbus Graham, *Two Letters Concerning the Early History (1817–1818) of the Medical College of Transylvania University Lexington, Kentucky.* ed. J. Hill Hamon. (Frankfort: Whippoorwill Press, 1993). I would like to thank Prof. Hamon for sending me a beautifully hand-printed and bound copy of his book. See also Robert Peter, M.D., *History of the Medical Department of Transylvania University.* Filson Club Publications No. 20 (Louisville: John P. Morton, 1905), 24–25. Kentuckiana Digital Library.
26. *Letters*, ed. Hamon; excerpt in Peters, p. 33, note.
27. 2 Feb 1876 in *Ibid.*, ed. Hamon p. 13–15. Note that Rafinesque, probably the best scientist on the faculty, never became a medical doctor because he had an aversion to dissection. For some historical context of Rafinesque and Transylvania in the medical botany movement, see Michael Flannery, "Medical Botany in Kentucky, 1792–1910," Transactions of the Kentucky Academy of Science 60 (Spring 1999):21–23.
28. 12 Feb 1876 in *Ibid.*, p. 20.
29. David Burrell, "Origins of Undertaking: How Antebellum Merchants made Death their Business," 9 Jun 1988. Internet.
30. *Letters*, ed. Hamon, p. 13–15.
31. David Burrell, "From Sanctity to Property: Dead Bodies in American Society and Law, 1800–1860," 1997. Internet. He cites this case from Margaret M. Coffin, *Death in Early America: the History and Folklore of Customs and Superstitions of Early Medicine, Funerals, Burials and Mourning* (1976). Coffin is mistaken in saying they had to pay a dollar in damages,

since Graham says the fine was one cent. One cent would be more in keeping with the money poor students would have available for such a purpose, and with the intention of the judge in awarding the damages.

32. It is interesting to note in this respect that in the South sometimes the bodies of slaves were made available for anatomical study—with the approval of the master. In 1834, according to the *Kentucky House Journal* (1833–1844), p. 104, there was a bill "to authorize and require the judge of the different Circuit Courts to adjudge and award the corpses of Negroes, executed by sentences of said judges, to the Faculties of the different chartered Colleges of the state, for dissection and experiment." It was rejected by the House of Representatives 41–34. (Wright, *Transylvania*, p. 85.) There is also a case which may serve to show how anxious students were for cadavers. In 1858 a Kentucky slave, soon to be hanged for murder, sold his body to two medical students for ten cents worth of candy. The state, which had paid $900 for the slave, presumably did not object. (Prof. A. M. Yealey, "The Story of Joe, a Slave Boy: A Story of Murder and Near Mob Violence," *The Northern Kentucky News*, 8 Oct 1954, p. 2).
33. Christopher Columbus Graham to Henry Clay (31 Aug 1825). *The Papers of Henry Clay*, ed. J. F. Hopkins, Vol. 4, p. 608.
34. George H. Daniels, *Science in American Society: A Social History* (New York: Knopf, 1971), p. 130–131.
35. Nye, p. 252.
36. Graham to Clay (31 Aug 1825). *The Papers of Henry Clay*, Vol. 4, p. 609. For a lengthy discussion of Rafinesque's incursion into the world of banking, see Leonard Warren, *Constantine Samuel Rafinesque: A Voice in the American Wilderness* (Lexington: University Press of Kentucky, 2004), "The World of Finance and Banking," p. 100–108. Warren concludes that by the time he left Kentucky, Rafinesque "suffered serious mental derangement." (p. 108)
37. Constantine S. Rafinesque, *A Life of Travels and Researches in North America and South Europe, or, Outlines of the Life, Travels and Researches of C. S. Rafinesque: Containing his travels in North America and the south of Europe, . . . &c., from 1802 to 1835—with sketches of his scientific and historical researches, &c.*.(Philadelphia: Printed for the Author, 1836), p. 74. Mrs. McDowell was the widow of Dr. Ephraim McDowell (1771–1830), a famous Kentucky surgeon. He was noted for his success in lithotomy, and in 1809 he performed the first ovariotomy.
38. Lewis Collins, *Collins' Historical Sketches of Kentucky*. (Frankfort: Kentucky Historical Society, 1966).
39. Frederick, Hall, *Letters from the East and from the West* (Baltimore: F. Lucas, Jr., 1840), p. 137. American Memory Project.
40. *Philosophy of Mind*, p. 215.
41. For more on Rafinesque, see John W. Thieret and David M. Brandenburg, "Rafinesque and Us," *Lloydiana*, Vol. 6, No. 1 (2001): 4–9. This may be accessed at: *http://www.geocities.com/kentuckyhist/rafine-thieret.html/*
42. R. C. Riebel. *Louisville Panorama: A Visual History of Louisville*. (Louisville: Liberty National Bank and Trust Company, 1954), p. 172. Of the $5,900,000 raised, the library received only $450,000. Where the rest of the money went is still a mystery.
43. I have not been able to unravel everything relating to the material placed in the Museum. The information relating to the disposition of the archaeological collection was given me by Joe Hardesty, of the Louisville Free Public Library. I would like to thank him in addition for providing me with copies of several valuable articles relating to Graham.
44. Knox followed Shaler as state geologist in 1880, though he had no geological training. He was fired by Gov. John Y. Brown I for not hiring the governor's son on the geological survey, but was offered a position in Washington, D.C. soon after.
45. Jillson apparently was not aware of this excavation, but otherwise provides the best summary of the digs in the 1800s. See Willard Rouse Jillson, *Big Bone Lick: An Outline of Its History, Geology And Paleontology*. (Louisville: The Standard Print. Co., 1936).
46. Christopher Columbus Graham, "The Mammoth's Graveyard," *Courier-Journal*, 29 Jan 1877, p. 1. This was reprinted other places, including the *Boone County Recorder*, 22 Feb 1877, p. 1. This may be accessed at: *http://geocities.com/bigbonehistory/graham-excavation.html/*
47. Cambridge University Library Darwin Project. The citation for these letters is: DAR 165:83–84. I have not been able to secure copies of these letters due to the prohibitive cost of getting them copied.
48. *Letters from the East and from the West*, p. 136. *Goût* means "taste, flavour, relish."
49. See Christopher Columbus Graham, "Pioneer Life: A Sketch of the Life and Services of Bland Ballard. Some Secrets of the Burr Conspiracy," *Louisville Monthly Magazine* Vol. 1, No. 1 (Jan 1879): 10–14; see also Allen's *History*.
50. Jackson to Thomas P. Moore. Letter published 2 Oct 1826 and cited in *Clay Papers*, 609.
51. Letter of Pres. Jefferson Davis to Graham Centennial Committee, 4 Oct 1884. Printed in Prof. A. M. Stickles, "The Christopher Columbus Graham Dinner," *Courier-Journal*, 1 Jun 1935.
52. *Philosophy of Mind*, p. 191–192. This has not been independently confirmed, but it is assumed Graham would not have made such a claim as there were still a number of people alive, including Jefferson Davis, who could have contradicted it if it were not so. Black Hawk wrote an autobiography and states they were returned home in a steamboat. Graham is not mentioned, but the dates fit.

53. Allen, p. 314. Daniels, p. 181, discusses the importance of the Railway Surveys for American Science.
54. The title printed on the spine of the original binding is "The True Science of Mind."
55. The catalogue of the Draper Collection lists a number of letters from Graham. *Calendar of the Kentucky Papers of the Draper Collection of Manuscripts,* ed. M. C. Weaks, (Madison: State Historical Society of Wisconsin, 1925; rpt. 1979).
56. W. O. McIntyre, "Memories of Dr. Christopher Columbus Graham," *Courier-Journal,* 27 Dec 1931.
57. Reuben T. Durrett, *The Centenary of Louisville.* (Louisville: John P. Morton, 1893). Kentuckiana Digital Library.
58. *http://newsarch.rootsweb.com/th/read/KYMERCER/2003-08/1060810882*
59. The unknown belle was Mollie Black, Tazewell, Tennessee. Corinne Roosevelt Robinson, a minor poet and sister of the President, visited the locale and wrote a poem about the incident. An interesting historical account of this tragedy is in Clark, *The Kentucky,* p. 220–222.
60. Another interesting memorial to Graham which has survived is on the Lincoln memorial in Springfield, Illinois. His image is engraved on the Kentucky panel of the monument. He was present at the marriage of Lincoln's parents and (according to reports) visited the family often. His last act was to dictate a letter to Lincoln's son Robert, then Secretary of War. For further information see: *http://www.nps.gov/libo/sculptured_panels3.htm/* and *http://www.ehistory.com/uscw/library/books/lincoln006.cfm/.* An early biographical account of Graham I neglected to mention above is: *The Biographical Encyclopedia of Kentucky* (Cincinnati: Armstrong and Company, 1878), p. 439.

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NOTES

Notes on root suckers and leaves of Kentucky Coffeetree (*Gymnocladus dioicus*; Fabaceae) in Kentucky.—*Root suckers.* Although the eastern North American Kentucky coffeetree (*Gymnocladus dioicus*; Fabaceae) (KCT) is a part of many floras and guides to wild or cultivated woody plants, many of these works do not mention that the tree can produce root suckers (root shoots), sometimes abundantly, a characteristic not highly desirable in a street or lawn tree. Even some of the best-known, standard horticultural works (e.g., Bailey [1] and Dirr [3]) do not discuss the suckers. Among the exceptions to the silence is Anonymous (4): "When the tree is cut down the roots send up a large number of suckers." Elwes and Henry (5) wrote, "The tree is noted in America for its habit of suckering from the roots when it is cut down. After a tree is felled the ground around to a distance of often 100 feet becomes filled with numerous suckers." Suckering, however, is not only a post-aboveground-mortem phenomenon. *Living* trees, too, may sucker. That these root shoots are a main means of spread of the tree was stated by Wilbur (6) and implied by Robertson and Lee (7); Steyermark (8) wrote, "The tree occurs sometimes in small colonies of rather widely separated individuals, resulting from the habit of the species of sending up root suckers at some distance from the parent tree." In Canada, where KCT occurs only in extreme southern Ontario, it rarely forms seeds but does produce root suckers. The trees, "at risk" in Canada, are used as nest sites by the increasing population of cormorants in the area. Many trees and suckers have been killed by the acidic droppings of the large number of birds (9).

This note reports on suckering of a staminate KCT on home grounds in Alexandria, Kentucky, during summers 2002, 2003, and 2004.

Suckering began in 2002 while the tree was still very much alive; it was 19 years old and had attained a height of 6.4 m and a DBH (diameter breast height) of 21 cm. Thirteen suckers developed in summer 2002, some as much as 6.8 m away from the parent plant. In early summer 2003 the tree was cut down, but suckering continued and accelerated. Thirty-five additional suckers had appeared by leaf fall 2003. The rate of suckering was even more impressive in 2004. By the time of this writing (16 Sep 2004) total production of suckers during the 3-year period was 220. They had indeed become a pest—perhaps even a plague—in the home garden.

Over the 3 years, most suckers were soon removed; eradication continues. Growth of the suckers was rapid. One, appearing in 2002, was left for 2 years; in 2004 it was taken down when it was 2.4 m tall, unbranched, and 2.5 cm in stem diameter.

Total production of suckers from the Alexandrian tree through mid-September 2004 was equivalent to ca. 7300 per acre, a datum that will change as more suckers appear. Although this may seem impressive, other trees can produce suckers at a much greater rate, e.g., trembling aspen (*Populus tremuloides*), up to ca. 30,000 suckers per acre (2).

Such abundant production of KCT suckers occurs elsewhere, too, as around a healthy staminate tree in a home yard in Middletown, Ohio; if the suckers there had not been removed, the yard would have been a "coffeetree thicket" (David M. Brandenburg pers. comm. 2003). Suckering is not confined to staminate trees: a living pistillate tree in Boone County, Kentucky, produces them (Richard Feist pers. comm. 2004).

KCT has been known as a wild-growing plant and as a cultivated tree for at least 250 years. Why, then, is the suckering habit so often not noted in literature and by some individuals who work with trees? Are only certain KCT genotypes prone to suckering? Under what conditions does KCT suckering occur? Is it a trait expressed only after a concatenation of certain environmental conditions? Does the removal of suckers within a day or two of their appearance contribute somehow to their continued production? These are questions yet to be answered.

In eastern North America other trees, too, produce suckers. Well known among these are tree-of-heaven (*Ailanthus altissima*), papaw (*Asimina triloba),* hackberry (Celtis occidentalis), trembling aspen (*Populus tremuloides*), black locust (*Robinia pseudoacacia*), and sassafras (*Sassafras albidum*).

Leaves. In common with juvenile leaves of various other trees, those of KCT suckers differ from mature leaves (Figure 1). On sucker shoots and seedlings of *KCT* the first one to nine leaves are usually 1-pinnate, 6 to 45 cm long, and with 3–12 pairs of pinnae. Later growth produces an increasing number of pinnately compound pinnae, starting with often one in a median position on the rachis of an otherwise 1-pinnate leaf. On some leaves a simple pinna or two may be produced between compound pinnae.

At the tip of primary and secondary rachises of the leaves of KCT may be an inconspicuous, soft bristle. Those I have seen do not exceed 1 cm, but Halsted (10), calling them "tendrils," reported that those on the primary rachis may reach 2.5 cm. What the bristles represent is uncertain, although they may simply be extensions of the rachis or "degenerate terminal leaflets" (5) much as can be seen in black walnut (*Juglans nigra*; Juglandaceae). Similar bristles may occur also on leaves of honey-locust (*Gleditsia triacanthos*; Fabaceae), a tree related to KCT.

Though frequently described as lacking, the stipules of KCT, when present, are represented by caducous, minute scales or bristles. Stipels are similar to stipules and, like them, are not always present.

Specimens vouchering this note are deposited in the herbarium of Northern Kentucky University (KNK).

I thank David M. Brandenburg, The Dawes Arboretum, Newark, OH, and Richard Feist, Burlington, KY, for data.

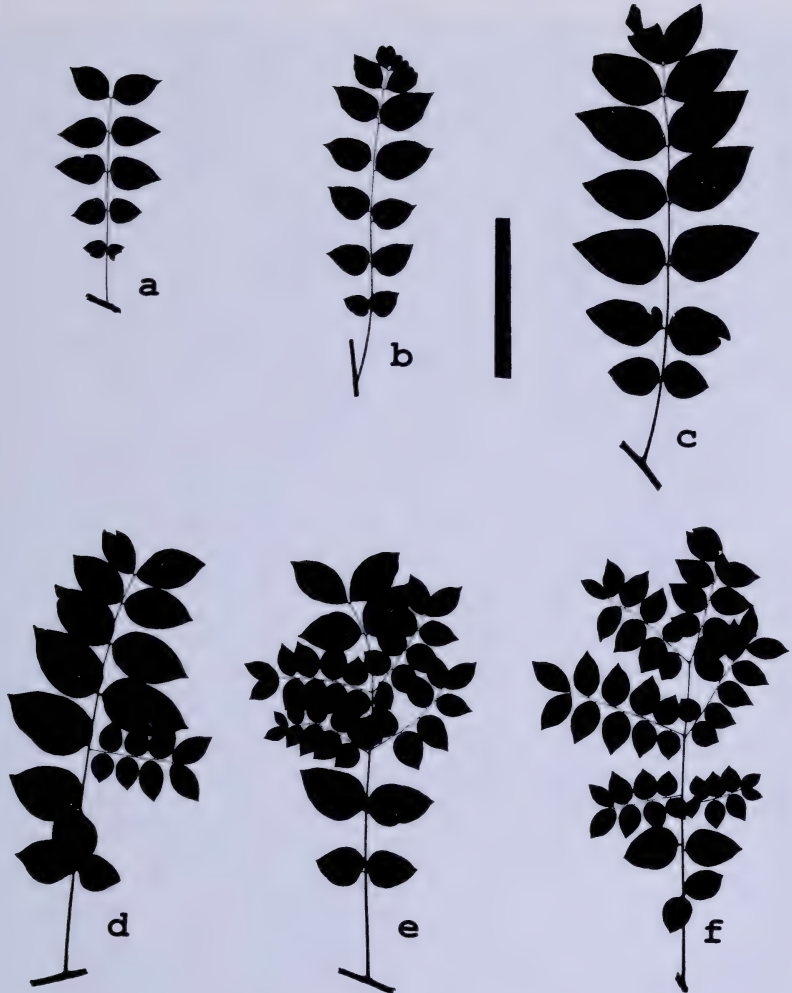

Figure 1. Silhouette of selected leaves from a Kentucky coffeetree sucker with nine leaves, showing change in leaf morphology from the base to the tip of the sucker. Omitted are leaves 3, 5, and 7, each of which was closely similar to the leaf immediately below it. (a) Leaf 1, one-pinnate. (b) Leaf 2, one-pinnate. (c) Leaf 4, one-pinnate. (d) Leaf 6, mostly one-pinnate but with a median pinna compound. (e) Leaf 8, with four median pinnae compound. (f) Leaf 9, mostly two-pinnate, with all pinnae but the basal two pairs compound, the leaf with essentially the same morphology as mature leaves of the species. The vertical bar = 10 cm.

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Vegetative Proliferation in *Eragrostis minor* (Little Lovegrass; Poaceae).—Unusual or malformed plant parts are occasionally seen in many groups of plants. These distortions, termed teratological, may occur in vegetative structures, such as leaves or stems, or in reproductive structures, that is, flowers and fruits (1, 2). Darwin (3) discussed morphological deviations in his *Origin of Species,* calling them "monstrosities," which he thought would be injurious or at least not useful, stating that such could grade into "varieties" if the condition was inherited by subsequent generations.

The replacement of various parts in the spikelets of grasses by vegetative growth has been known at least since it was reported in 1620 by Bauhin (4) in the species now known as *Poa bulbosa* L., and in 1690 by Ray (5) in what is now known as *Festuca vivipara* (L.) Sm. Many other species of grasses have been reported to exhibit similar conditions. In a synopsis paper on the subject, Beetle (6) reported such replacement of parts in about 80 different grass species, many of which have been given varietal or form epithets such as "vivipara," "prolifera," or even "monstrosa." A fairly widely distributed example is *Poa bulbosa* L. (bulbous bluegrass), known by most people only from its proliferative form, *P. bulbosa* ssp. *vivipara* (Koel.) Arcangeli, though a non-proliferative form is sometimes seen (7).

Beetle (6) recognized three types of vegetative structures in grass inflorescences: *proliferation,* in which the paleas and lemmas are replaced by leaf-like structures; *phyllody,* in which the paleas and lemmas are replaced with such well-developed leaves that they are differentiated into sheaths and blades; and true *vivipary,* in which a seed germinates precociously on the parent plant to give rise to a new individual (8). The terms vivipary and proliferation have been used almost interchangeably and without discrimination in the literature for several hundred years, but Arber (9) argued that the term "vivipary" should be restricted to conditions where seeds germinate and grow in situ.

The causes of vegetative proliferations in grasses are variable, and may include one or several of the following (6, 10, 11, 12, 13): heritable causes, such as hybridization and polyploidy; malformations (teratology), caused by mechanical injury, chemical damage, or attacks by pathogens or pests; and adverse environmental conditions, such as in water levels, light levels, high altitude or latitudes, and abrupt changes in day length or temperature.

Vegetative proliferations have been reported for several species of the genus *Eragrostis* (love grasses). Beetle (6) listed three species of the genus in which the condition is known: *E. brizoides* (L.f.) Nees, *E. capensis* (Thunb.) Trin., and *E. virescens* Presl; in addition, proliferations were reported by Jain (14) in *E. angetica* (Roxb.) Steudel. I have recently seen several specimens of *Eragrostis minor* (from Kentucky, Michigan, and Ohio; see Specimens Examined) with abnormal inflorescences, and since I was unable to uncover any literature record of such a condition in this grass species, further investigation was warranted.

Eragrostis minor Host (Little lovegrass; Poaceae; Fig. 1A, 1B), also known as *Eragrostis poaeoides* Beauv. ex Roemer & J. A. Schultes, is a widely distributed, weedy species introduced from Europe. It is found in nearly all of the lower 48 states in the United States (http://plants.usda.gov/), often along railroad tracks (15, 16), or roadsides and paths (17). It is similar to *E. cilianensis* (All.) Janchen (stink grass; = *E. megastachya* (Koel.) Link), from which it differs by having lemmas 1.5–2 mm long (2–2.8 in *E. cilianensis*), having anthers 0.2 mm long (0.5 in *E. cilianensis*), and often lacking glands on the keel of the lemma (usually present in *E. cilianensis*) (18, 19).

The proliferations in spikelets of *E. minor* (Fig. 1C) fit best the definition of "phyllody" as given by Beetle (6). In some spikelets, all parts, including glumes, have become leaf-like, the smaller appearing as blades, and the larger differentiated into sheaths and blades. Some spikelets have only a single floret replaced by a proliferation, while other spikelets are completely replaced by what appears to be a plantlet. In these proliferative structures, no sexual parts are present even though remaining florets appear normal and contain anthers and/or pistils. It is unknown whether the vegetative proliferations in *E. minor* are able to function as propagules.

Many plant species along the railroad tracks at the Kentucky and Ohio sites (see Specimens Examined) showed evidence of herbicide treatment. It is possible that this is the cause of the unusual morphology of some spikelets from these populations, though this remains to be tested.

Specimens examined. KENTUCKY: Mason County; Marysville; weedy in railroad yard, 3 Jul 2001, *M. A. Vincent 9562, J. W. Thieret, & W. M. Vincent* (KNK, MU). MICHIGAN: St. Joseph County; Three Rivers; weedy ground at railroad crossing, 17 Aug 1995, *A. W. Cusick 32692* (MICH, MU). OHIO: Butler County; Oxford; weedy along railroad line, 19 Jul 2004, *M. A. Vincent et al. 12159* (DAV, DOV, ISC, KNK, MO, MICH, MU, OSH, US, UTC).

I am grateful for assistance from the following people in this study: Thomas Lammers (OSH), Shane Shaw (MU), and John W. Thieret (KNK).

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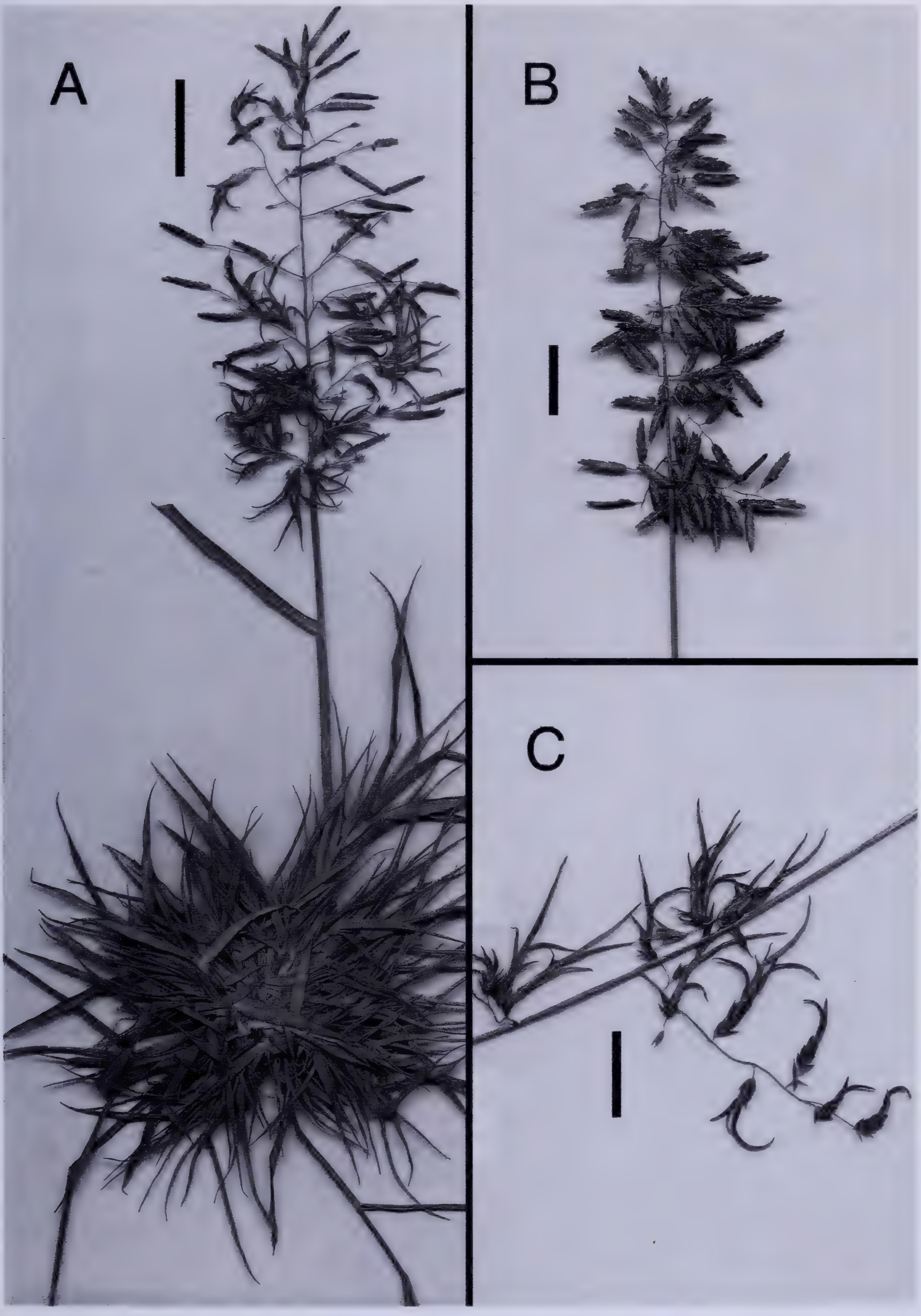
Figure 1. A. Whole plant of *Eragrostis minor,* showing inflorescence with mixed normal and proliferative florets (*Vincent 12159,* MU; scale bar = 2 cm). B. Inflorescence showing normal florets (*Vincent 12159,* MU; scale bar = 1 cm). C. Portion of an inflorescence showing proliferative florets (*Cusick 32692,* MU; scale bar = 1 cm).


A
B
C

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J. Ky. Acad. Sci. 65(2):159–162. 2004.

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Guidelines for Contributors to the Journal

1. GENERAL

A. Original research/review papers in science will be considered for publication in JKAS; at least the first author must be a member of the Academy. Announcements, news, and notes will be included as received.

B. Acceptance of papers for publication in JKAS depends on merit as evaluated by each of two or more reviewers.

C. Papers (in triplicate) may be submitted at any time to the editor.

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List in the cover letter your telephone/FAX numbers, your E-mail address, and the names, addresses, and telephone numbers of two persons who are potential reviewers.

D. Format/style of papers must conform to these guidelines and also to practices in recent issues of JKAS, which are, in effect, a style manual.

E. Papers should be submitted in hard copy. Do not staple pages together.

F. Indent the first line of each paragraph (but not the first line of entries in the Literature Cited).

2. FORMAT

A. Papers should be in 12-point type on white paper 8.5 × 11 inches, with margins at least 1 inch all around. Double-space throughout the paper (i.e., one full line of space between each two lines of text, literature cited, or tabular data). Do not justify right margins.

B. Except for scientific names of genera and of infrageneric taxa, which should be typed in italics, the same type (roman) should be used throughout (i.e., one type size only; bold only for paper title).

C. Sequence of sections in papers should, where appropriate, be as follows: title of paper, name/address of author(s), abstract, body of paper, footnotes, table captions, figure captions (all the preceding on consecutively numbered pages), tables, and figures.

D. The running head (top right) should give name(s) of author(s), a short version of paper title, and page number of total.

E. The first page should include the running head and, centered near the top of the sheet, the paper's title and the name and address of author(s). These should be followed immediately by the abstract. (The first page should look as much as possible like the first page of articles in JKAS.)

F. The abstract, not to exceed 200 words, should be concise, descriptive, and complete in itself without reference to the paper.

G. The body of the paper should, where appropriate, include the following sections: Introduction, Materials and Methods, Results, Discussion, Summary, Acknowledgments, and Literature Cited.

H. No more than three levels of headings should be used: level 1, in capitals, centered; level 2, in capitals/lowercase, flush left; level 3, in italics, a paragraph indent, with initial capital only (except proper nouns and adjectives), and followed by a period, the text then starting after one blank space.

I. Personal communications (avoid if possible) should be indicated in the text as follows: (name, affiliation, pers. comm., date), e.g., (O.T. Mark, Wainwright College, pers. comm., 5 Jun 1995).

3. STYLE

A. In text, spell out one-digit numbers unless they are used with units of measure (four oranges, 4 cm) and use numerals for larger numbers; do not begin any sentence with a numeral.

B. Use no footnotes except those for title page and tables. Footnotes, identified by consecutive superscript numbers, should be entered on a separate sheet.

C. Measurements should be in metric and Celsius units. Define lesser-known symbols and give the meaning of acronyms at first use. Express time of day in the 24-hour system. Dates should be written day, month (abbreviated to three letters), year without internal punctuation. Units with multiple components should have individual components separated by a virgule (e.g., g/m^2 or $g/m^2/yr$).

D. Names of authors of binomials may be included but only at the first mention of the binomial. Cultivar names are not italicized but are enclosed in single quotes.

E. Useful guides for contributors to JKAS are the following: *Scientific style and format: the CBE manual for authors, editors, and publishers,* 6th ed., Cambridge University Press, 1994; *The Chicago manual of style,* 14th ed., University of Chicago Press, 1993; *The ACS style guide,* American Chemical Society, Washington, DC, 1986; and *AIP style manual,* American Institute of Physics, New York, 1990.

4. IN-TEXT CITATION OF LITERATURE

A. Cite publications in the text by author(s) and date—e.g., (Readley 1994); multiple citations should be in alphabetical order and separated by semi-colons—e.g., (Ashley 1987; Brown 1994; Foster 1975); multiple citations of works by one author(s) should be in chronological order—e.g., (Jones 1978, 1983); publications by one author(s) in the same year should be distinguished by a, b, c, etc.—e.g., (Smith 1994a, 1994b). For in-text references to works with one or two authors use names of both authors—e.g., (Jones and Williams 1991); for works with three or more authors use name

of the first author followed by et al.—e.g., (Lee et al. 1985).

B. Do not include any reference unless it has been published or accepted for publication ("in press"; see below).

5. LITERATURE CITED

A. List all authors of each entry. Do not abbreviate journal titles; abbreviations for these will be supplied by the editor.

B. The first line of each reference should be typed flush left; the remaining lines should be indented.

C. Examples of common types of references are given below.

JOURNAL ARTICLE

Lacki, M.J. 1994. Metal concentrations in guano from a gray bat summer roost. Transactions of the Kentucky Academy of Science 55:124–126.

BOOK

Ware, M., and R.W. Tare. 1991. Plains life and love. Pioneer Press, Crete, WY.

PART OF A BOOK

Kohn, J.R. 1993. Pinaceae. Pages 32–50 in J.F. Nadel (ed). Flora of the Black Mountains. University of Northwestern South Dakota Press, Utopia, SD.

WORK IN PRESS

Groves, S.J., I.V. Woodland, and G.H. Tobosa. n.d. Deserts of Trans-Pecos Texas. 2nd ed. Ocotillo Press, Yucca City, TX.

6. ILLUSTRATIONS

FIGURES (LINE DRAWINGS, MAPS, GRAPHS, PHOTOGRAPHS)

Figures must be camera-ready, glossy, black-and-white prints of high quality or laser prints of presentation quality. These should be designed to use available space effectively: a full page or part of one, or a full column or part of one. They should be mounted on heavy white board and covered with a protective sheet of paper; photographs to be grouped as a plate should have no space between them. Dimensions of plates must observe page proportions of the journal. Each illustration in a plate may be numbered as a separate figure or the entire plate may be treated as one figure. Include scale bars where appropriate. Lettering should be large enough to be legible after reduction; use lowercase letters for sections of a figure. Figure captions should be self-explanatory without reference to the text and should be entered on a page separate from the text. Number figures in Arabic numerals. Statistics presented in figures should be explained in the caption (e.g., means are presented ± SE, n = 7).

TABLES

Each table and its caption must be double-spaced, numbered in Arabic numerals, and set on a sheet separate from the text. The caption should begin with a title relating the table to the paper of which it is a part; it should be informative of the table's contents. Statistics presented in the table should be explained in the caption (e.g., means are presented ± SE, n = 7). Table should be submitted in hard copy only; they need not be included on a disk.

7. ETHICAL TREATMENT OF ANIMALS AS RESEARCH SUBJECTS

If vertebrate or invertebrate animals are involved in a research project, the author(s) should follow those guidelines for ethical treatment of animals appropriate for the subjects, e.g., for mammals or for amphibians and reptiles. Papers submitted to JKAS will be rejected if their content violates either the letter or the spirit of the guidelines.

8. PROOFS

Authors are responsible for correcting proofs. Alterations on proofs are expensive; costs will be assessed to authors. Proofs must be returned to the editor within 3 days after the author receives them; delay in return may result in delay of publication.

9. REPRINTS

Forms for ordering reprints will be sent to the author when the proofs are sent. They are to be returned directly to Allen Press, not to the editor.

10. PAGE CHARGES

Pages charges are assessed to authors of papers published in *Journal of the Kentucky Academy of Science.*

11. ABSTRACTS FOR ANNUAL MEETINGS

Instructions on style of abstract preparation for papers presented at annual meetings may be obtained from the editor. Copies will be available also at each annual meeting of the Academy.

NEWS

Kentucky Heritage Land Conservation Fund

The mission of the Kentucky Heritage Land Conservation Fund (KHLCF) is to award funding to purchase and preserve selected natural areas in the Commonwealth; to protect rare and endangered species and migratory birds; to save threatened areas of natural importance; and to provide natural areas for public use, outdoor recreation, and education.

Established by the 1994 Kentucky Legislature, KHLCF is administered by a 12-member board appointed by the governor. The board can award grants to acquire and protect areas of natural significance to local governments, state colleges/universities, and specified state agencies. Special consideration will be given to the funding of agencies working together to meet the listed goals. All acquisition applications, along with comprehensive management plans, must be submitted to and approved by the board.

The year 2003 was another eventful year for the KHLCF board. During 2003 it received and reviewed a large number of applications and approved 14 projects in 13 counties. In 2003 local government projects were approved in Calloway, Clark, Green, Harrison, Livingston, Logan, and Oldham counties; state agency projects were approved in Franklin, Garrard, Hardin, Harlan, Larue, and Letcher counties. The board also approved the Pine Mountain Trail State Park and provided initial acquisition funds. The trail will traverse Bell, Harlan, Letcher, and Pike counties.

Since the first awards were made in October 1995 the board has approved 116 projects in 56 Kentucky counties. Almost 20,000 acres have been purchased.

The fund is supported by the state portion of the unmined minerals tax, environmental fines, the $10 additional fee to purchase a Kentucky nature license plate, and interest on the fund's assets.

For more information, contact the Department of Natural Resources, Commissioner's Office, 663 Teton Trail, Frankfort, KY 40601. The phone number is (502) 564-2184; the e-mail address is www.heritageland.ky.gov.

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